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An assessment of cognitive and sensorimotor deficits associated with appsw and p301l mouse models of alzheimer's disease

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AN ASSESSMENT OF COGNITIVE AND SENSORIMOTOR DEFICITS ASSOCIATED WITH APPsw AND P301L MOUSE MODELS OF ALZHEIMER'S DISEASE

by

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ABSTRACT

Behavioral characterization of animal models for Alzheimer's Disease is critical for the development of potential therapeutics and treatments against the disease. While there are several known animal models of AD, three current models--APPsw, P301L, and APPsw+P301L--have not been well characterized, if at all. This study, therefore, aimed to perform a full behavioral characterization of these three models in order to better understand the impairments associated with each one. Between 5 and 8.5 months of age, animals were behaviorally tested in a variety of sensorimotor, anxiety, and cognitive tasks. The number of tau+ neurons in the forebrains of P301L mice was then compared to their behavioral performance.

Results of this study indicate that retinal degeneration (rd) seriously impairs the performance of mice in behavioral tasks. Animals that carry the homozygous allele of this mutation must, therefore, be eliminated from any such study requiring visual acuity. After this elimination, my findings indicate that APP mice are impaired in several cognitive tasks (including platform recognition, Morris maze, Y-maze, and radial-arm

water maze) at a young early age (5 to 8.5 months of age). These mice have fairly normal sensorimotor function, showing significant impairment only in balance beam performance starting at 5 months. Although P301L mutant Tau mice, as a group, did not have significant impairments in any sensorimotor or cognitive task, correlation analysis revealed that higher numbers of tau+ neurons in cortex and hippocampus were associated with poorer cognitive performance. Finally, discriminant function analysis (DFA) appears able to accurately discriminate between the three transgenic groups of mice using only an 8-measure data set.

In conclusion, this study provides the first comprehensive, multiple task behavioral assessment of the APPsw and P301L animal models of AD, indicating that APPsw mice are cognitively impaired at an early age while P301L mice are largely unimpaired through 8.5 months. Nonetheless, correlational analysis implicates the formation of neurofibrillary tangles in the onset of cognitive impairments. Finally, my findings recommend the continued use of DFA to determine if groups of animals, based on different transgenicity or therapeutic treatment, could be discriminated between from their behavior alone.

Introduction

Alzheimer's disease (AD) is widely considered to be the most prevalent form of dementia, occurring primarily in mid to late-life. Statistics indicate that AD affects up to 10% of all individuals over the age of 65 and 40% of those over the age of 80. Overall, AD accounts for approximately 81% of all cases of dementia. As life expectancies continue to increase worldwide due to advances in medical treatment, the number of AD cases is expected to rise dramatically over the next 50 years (Janus and Westaway, 2001). Therefore, the development of therapeutics to combat AD and alleviate its symptoms is becoming more critical with each passing year.

AD is characterized by progressive memory impairment, disordered cognitive function, and a progressive decline in language function. The time-course for AD in affected individuals can last between 2 to 20 years. Typically, the first symptoms related to AD are moderate losses in working memory and language function accompanied by mild depression. As the disease progresses, the deficits in working memory and language function become more severe and long-term memory begins to be affected. Lastly, the final stages of AD are characterized by a virtually complete loss of memory and intellect, leaving the patient in a bedridden, vegetative state that ultimately results in death.

Researchers have already identified what many refer to as the two "pathological hallmarks" of AD—the formation of neuritic plaques and neurofibrillary tangles. Neuritic plaques are small patches composed of amyloid protein that sticks to neurons and attracts

microglia as well as astrocytes. The formation of these plaques leads to neuronal death, primarily in areas of the limbic and association cortices. Neurofibrillary tangles (NFT's) are created when tau, a normal, microtubule-associated protein found in most neurons, becomes hyperphosphorylated to form paired helices that disrupt axonal conduction and, ultimately, results in the death of the neuron. NFT's can be found in the entorhinal cortex, hippocampus, parahippocampal gyrus, amygdala, frontal, temporal, parietal, and occipital association cortices of AD patients. While these two lesions represent the hallmark pathologic characteristics of AD, neuritic plaques and NFT's can occur independently of each other in AD as well as in other, less common neurodegenerative diseases (Selkoe, 2001).

Amyloid Precursor Protein

As previously mentioned, neuritic plaques, one of the two hallmarks of AD pathology, are composed primarily of amyloid protein that becomes deposited in the brain. Specifically, it is β-amyloid, or Aβ, that is principally responsible for the formation of these neuritic plaques. Aβ, a protein composed of either 40 or 42 amino acids is, itself, synthesized from a much larger parent protein named amyloid precursor protein, APP. APP is a protein comprised of 695-751 amino acids that is commonly found in the membranes of neuronal cells throughout the brain and central nervous system (Selkoe, 2001). The APP protein contains three principal sites where proteolytic enzymatic cleavage can take place that is critical to the ontogeny of AD. Cleavage by α-secretase at amino acid 687 results in two harmless APP subunits, APP_s-α and C83. Cleavage of APP

at amino acids 671 and 711/713 by β-secretase and γ-secretase, respectively, results in the release of the cytotoxic $A\beta_{40}$ and $A\beta_{42}$ peptides, which are deposited.

Generally, it is thought that $A\beta_{42}$ begins the process of plaque development by forming loose aggregates (diffuse deposits). This complex later attracts $A\beta_{40}$, forming a much denser, more compact plaque (Selkoe, 2001). As Aβ is deposited and begins to form fibrils, microglia, the brain's phagocytic cells, are attracted to the plaque site and become activated. These activated microglia, which carry receptors for Aβ, are thought to assist in clearing Aβ from brain tissue (Yan et al., 1996; Ard et al., 1996). However, activated microglia are a two-edged sword in that they also respond to Aβ-activated complement by secreting toxic agents such as free radicals and harmful proteases. (Rogers et al., 1992). Activated microglia also release cytokines, such as IL-1 and TGF-β, which serve to activate astrocytes and attract them to the locations of Aβ plaques. These activated astrocytes then trigger the release of two inflammatory mediators, Apolipoprotein E (ApoE) and anti-chymotrypsin (ACT). ACT, an acute-phase inflammatory protein that is over-expressed in astrocytes that surround Aβ plaques, is thought to promote further deposition of Aβ and formation of more mature plaques through direct molecular interaction with Aβ (Nilsson et al., 2001). ApoE, a 299-amino acid lipid transport protein, is also thought to play a role in AD pathogenesis by mediating the conformational transition of Aβ into β-pleated sheets, which further promotes deposition (Holtzman et al., 1999; Wisniewski et al., 1992).

Researchers have come to understand that, as people age, levels of deposited brain Aβ increase, enhancing the risk of developing Alzheimer's Disease. While this increase

generally occurs in mid-life or later, some individuals experience an increase in Aβ at a much earlier age, leading to onset of AD symptoms. While the mechanisms that lead to this increase in brain Aβ are not fully understood and could involve age-related reductions in Aβ breakdown or clearance from the brain, certain mutations have been identified that are purported to enhance β-secretase and/or γ-secretase activity, increasing the likelihood that Aβ will result in the brain. One such mutation occurs on the presenilin-1 protein (PS-1) and has been thought to significantly increase the activity of γ-secretase (Selkoe, 2001). While the exact mechanism of PS-1 action with regards to γ-secretase has yet to be elucidated, two general theories have been developed to describe its potential function. Some researchers have suggested that PS-1 is a direct co-factor of γ-secretase and, thus, is required for the normal function of γ-secretase. This theory is supported by experiments showing that PS-1 "knock-out" mice have deficient γ-secretase function (De Strooper et al., 1998). The second theory is that PS-1, which serves as a membrane-bound protein, may have a role in cell trafficking, controlling the interaction of γ-secretase with its necessary cofactors. This theory is not widely supported (Naruse et al., 1998). Also, point mutations on the APP protein near the β-secretase and γ-secretase cleavage sites can alter the activity of these enzymes, resulting in greater $A\beta_{42}$ release (Selkoe, 2001). Cases of AD in which a genetic mutation is to blame for an increase in $A\beta_{42}$ release are referred to as familial AD, so called because it is inheritable through an autosomic dominance process and is thus passed down through families. Familial AD comprises only 10% of the known cases of AD in individuals. The majority of AD cases, ~90%, occur through unknown causes and are referred to as sporadic AD (Janus and Westaway, 2001).

Neurofibrillary Tangles

The second pathological hallmark of AD is the formation of neurofibrillary tangles within the axons of affected neurons in the brain. These tangles are composed of a protein named tau, which is normally bound to axon microtubules. When tau protein becomes hyperphosphorylated by unknown kinases, it dissociates from the microtubule and forms insoluble, paired, helical filaments. The formation of these paired, helical filaments strangles the axon, eventually killing it and preventing any downstream communication (Selkoe, 2001). Recently, it has been concluded that tangle formation can be induced by Aβ–mediated phosphorylation of the tau protein, propagating the theory that amyloid deposits and NFTs may act in concert to generate AD pathology (Götz et al., 2001). In addition to AD, NFT formation has also been linked with other less common neurodegenerative diseases such as frontal temporal lobe dementia, Kuf's Disease, and sclerosis panencephalitis (Selkoe, 2001).

Transgenic Mouse Models of Alzheimer's Disease

In order to better understand the mechanisms underlying AD and test potential therapeutics against the disease, transgenic mouse models are needed. Transgenic mouse models are generated by inserting either wild-type or mutant AD transgenes into the genome of a fertilized egg. Specific promoters, such as PDGF1-β (Games et al., 1995), PrP (Hsaio et al., 1996), and thy-1 (Sturchtery-Pierrat et al., 1997), are used to ensure that the desired mutation is expressed in the brain. When the litter is born, the progeny are genetically screened to select only the mice that carry the mutation. In this way, we may observe the effects of particular genes of interest in the pathophysiology of AD.

One particular mutation that results in an increased release of toxic $A\beta_{42}$ is the Swedish mutation (APPsw), so named because it was discovered in a large Swedish family that suffers from familial AD. The APPsw mutation is a double point mutation that occurs at amino acids 670 and 671 of the APP protein, where a lysine residue is changed to asparagine and methionine is replaced by leucine, respectively. The APPsw mouse model is generated using a hamster prior protein (PrP) promoter that expresses the mutation in neurons of the brain. The combination of these two mutations on the APP protein is thought to increase the activity of β-secretase, creating a greater likelihood that $A\beta_{40}$ and $A\beta_{42}$ will be released (Selkoe, 2001).

In addition to the APPsw mutation, the V717F point mutation on the APP gene, also called the PDAPP mutation, is known to cause an increase in $A\beta_{42}$ release. Transgenic mice that carry this mutation over express a mutated human APP minigene that is driven by the platelet-derived growth factor promotor. The result of this mutation is increased γ-secretase activity, which leads to greater cleavage at the carboxy terminus of the Aβ protein (Dodart et al., 2000).

As mentioned previously, mutations to the PS1 protein, which participates in the γ-secretase complex, also result in a greater release of $A\beta_{42}$, apparently by increasing the activity of γ-secretase (Selkoe, 2001). Two such mutations, M146L or M146V, are currently employed in transgenic mice, under control of the PDGF-β2 promotor. When either of these PS-1 mutations are combined with the APPsw mutation in transgenic mice, commonly called the PSAPP model, Aβ production is further enhanced (Duff et al., 1996; Holcomb et al., 1998), resulting in enhanced and earlier Aβ deposition.

Mutations to the tau protein can also promote AD pathology, notably NFT formation intraneuronally. In addition to AD, NFT formation prominently found in several other neurodegenerative disorders (collectively known as "tauopathies"): progressive supranuclear palsy (PSP), corticobasal ganglionic degeneration (CBD), and frontotemporal lobe dementia (FTD). FTD, the most common of these disorders, involves impaired social conduct, diminished speech leading to muteness, and progressive dementia in affected patients (Kwon, 2002). There are three such point mutations to the tau protein that are commonly used in transgenic mouse models of AD: P301L controlled by the mouse prion protein promotor (MoPrP), V337M, under the expression of the PDGF-β promotor, and R406W, under control of the CaMK-II promotor. All of these mutations are capable of generating NFT's when overexpressed in transgenic mice. Each of these models, however, differ in their temporal sequence and regional distribution of NFT formation, as will be discussed later.

In the following pages, I will attempt to summarize the current literature describing transgenic mice carrying these different mutations that produce Alzheimer's-like pathophysiology. In particular, I will be discussing the principle behavioral and pathological findings from these studies in order to provide a reasonable assessment of what is currently known in the field of Alzheimer's transgenics with regard to these mutations.

APPsw Model: Pathology and Brain Ab levels

The first study to examine the APPsw mouse model of AD was authored by Hsaio et al. (1996). In this study, the authors determined, through ELISA, that transgenic mice

between 11 and 13 months of age have, on average, 5 times more $A\beta_{40}$ in their brains than transgenic mice between the ages of 2 to 8 months. Likewise, the oldest group of transgenic mice was also found to have 14 times more brain $A\beta_{42}$ than mice in the 2-8 month-old group. Of the three Tg+ mice in the 11 to 13 month-old age group that underwent neurochemical analysis, all three were found to possess classic senile plaques, including dense amyloid cores, as well as diffuse deposits of Aβ. These deposits were limited to frontal, temporal, and entorhinal cortices, as well as the hippocampus, presubiculum, subiculum, and cerebellum. The densest amyloid cores were found in the cortex, subiculum, and presubiculum. Amyloid deposits were absent in all of the non-transgenic mice analyzed in this study, as well as APPsw mice in the 2-8 month age range.

A study conducted by Irizarry et al. (1997) found that Tg2576 mice at 16 months of age, bearing the APPsw mutation, possess extensive Aβ deposits in the molecular layer of the hippocampus, the cortex, and the amygdala. Specifically, the authors determined that the CA1 region of the hippocampus, dentate gyrus, cingulate cortex, and entorhinal cortex possessed an average amyloid burden of between 3.6 to 8.5%, as determined through an immunostain using the R1282 polyclonal antibody. As expected, none of the non-transgenic subjects demonstrated any Aβ immunoreactivity. Despite these moderate levels of Aβ in and around the hippocampus, the study concluded that Tg+ mice did not differ significantly from Tg- test subjects in the number of neurons in the CA1 region of the hippocampus. This finding is particularly interesting since the hippocampus undergoes extensive neuronal loss in the course of human AD and is thought to be associated with behavioral deficits in mice. These results suggest that behavioral deficits

incurred by APPsw transgenic mice might be the result of APP overexpression or the abundance of small Aβ assemblies, rather than hippocampal cell loss.

In a similar study, Calhoun et al. (1998) examined a group of 14-18 month-old APP23 mice expressing the Swedish mutation, but with a different promotor from Tg2576 mice. Like Irizarry et al. (1997), Calhoun et al. (1998) found that these mice possessed a large number of deposited Aβ plaques in the neocortex and hippocampus. According to the study, at least 90% of plaques contained dense, fibrillar amyloid cores, similar to the kind found in human cases of AD. In addition, vascular amyloid was detected, primarily in the meningeal, neocortical, and thalamic vessels. In contrast to the Irizarry et al. (1997) study, however, Calhoun et al. (1998) found that Tg+ mice showed a significant decrease in the number of CA1 neurons when compared to their Tg- counterparts. Specifically, the study indicated that Tg+ mice exhibited a 14% reduction in the number of CA1 neurons, with some animals possessing a particularly high plaque load losing up to 25% of CA1 neurons. In general, the authors found that CA1 neuron loss was directly proportional to Aβ plaque load. This finding challenges the observation made by Irizarry (1997), stating that no appreciable CA1 neuronal loss is evident in Tg+ mice possessing a high Aβ plaque load. In the neocortex, however, Calhoun et al. (1998) found no evidence of global neuronal loss and suggest that the neocortical neurons are simply being physically displaced.

A study by Frautschy et al. (1998) examined a group of 10-16 month-old Tg2576 mice bearing the APPsw mutation to observe the relationship between plaque formation and microglial activation. The study found that Tg+ animals possessed clusters of microglia surrounding Aβ deposits, while Tg- animals had no such microglial clusters. In

addition, it was observed that subjects containing small Aβ deposits showed only a few or no activated microglia, while those who had larger Aβ deposits had greater amounts of activated microglia. Further, the authors discovered that the $A\beta_{40}$ antibody was more successful at staining activated microglia than the $A\beta_{42}$ antibody, suggesting that $A\beta_{40}$, and not $A\beta_{42}$, may be primarily responsible for initiating the microglial response in the entorhinal and occipital cortex and the hippocampus. This theory is confirmed by evidence obtained in previous studies indicating that, by 11-12 months of age (the approximate time when microglia begin to show activation in the onset of AD), 60% of Aβ plaques in Tg2576 mice are composed of $A\beta_{40}$.

To identify the role of Aβ deposition in the formation of oxidative free radicals in the brains of mice, Pappolla et al. (1998) studied groups of Tg2576 mice at 4 months and 21-25 months of age. These mice were sacrificed and immunostained for markers of oxidative damage in the brain, specifically, CuZn superoxide dismutase (CuZnSOD) and heme-oxygenase-1 (HO-1). In addition, the authors also stained for Aβ and ubiquitin, using specific monoclonal antibodies. The authors found that, at 21-25 months of age, the Tg+ mice displayed extensive signs of oxidative damage colocalized to regions of the brain where Aβ plaques were found. Further, these regions of the brain with plaque staining and oxidative markers were also found to stain for ubiquitin, an indicator of free-radical-mediated cytotoxicity. In contrast, 4 month-old Tg2576 mice showed no signs of oxidative markers or Aβ plaques. Older Tg- mice, 21-27 months of age, likewise, failed to demonstrate any Aβ plaques or oxidative markers. These findings promote the theory that Aβ plaque deposition and free radical-induced cell death in the brain are temporally linked. An in vitro cell culture study performed by Pappolla et al. (1998) further

implicates Aβ in the formation of free radicals. In their experiment, the authors cultured PC12 cells in the presence of $A\beta_{25-35}$, and observed that these cells underwent rapid cell death and demonstrated the presence of oxidative markers.

Benzing et al. (1999) examined a set of ten Tg2576 mice carrying the APPsw mutation and found that they begin to show moderate amounts of Aβ deposits at 12 months of age and considerable deposition by 18 months. At the 18-month time point, most of these deposits occurred in the temporal cortex, where plaques covered 24% of the cortical surface area. Aβ plaques were also extensively found in the fronto-parietal lobe, where 16% of the surface area was covered in plaques, and the hippocampus, which demonstrated 10% surface coverage by plaques. Of the plaques found in the temporal and fronto-parietal lobes, approximately 3% was found to be compact in nature, while about 6% of the hippocampal plaques were similarly constituted. Interestingly, at the 12-month time point, the cortical surface coverage of Aβ plaques was considerably less, while the relative percentage that is compact stayed the same.

Through the use of immunostaining, Benzing et al. (1999) also discovered that the majority of fibrillar Aβ plaques in 12 and 18 month-old mice were closely accompanied by activated microglia, the macrophages of the brain. These activated microglia were found to contain IL-1β and TNFα, two immune cytokines. In addition, IL-6, another immune cytokine, was found in activated astrocytes surrounding fibrillar Aβ plaques at both the 12 and 18-month time point. These two findings, taken together, serve to confirm the role of the neuroinflammatory processes in the pathogenesis of human AD.

Another set of Tg2576 mice with the APPsw mutation was studied by Mehlhorn et al. (2000). In the study, the mice were shown to be free of fibrillar Aβ plaques in the

hippocampus and cerebral cortex until approximately 14 months of age, despite expressing the mutant APP mRNA as early as 2 months of age. Even at 14 months, however, the Aβ plaque load is minimal. Immunostaining of the brains of these mice revealed a large number of reactive astrocytes surrounding the newly developed fibrillar Aβ plaques in the cerebral cortex at the 14-month time point. These astrocytes were found to contain active forms of IL-1β and glial fibrillary acidic protein (GFAP), a marker for astrocytes. Immunocytochemistry performed on sections of cerebral cortex at the 12 and 13-month time points revealed no detectable IL-1β in Tg2576 mice, suggesting that fibrillar Aβ deposition is required for astrocytes to become activated. In addition, a multiple-probe ribonuclease protection assay performed on the frontal, parietal, occipital, and entorhinal cortices of 2-13 month-old Tg2576 mice failed to detect the induction of any pro-inflammatory cytokine mRNA, indicating the lack of any inflammatory response prior to Aβ deposition. These two findings seem to indicate that the deposition of Aβ and the formation of fibrillar Aβ plaques are needed to trigger the release of pro-inflammatory cytokines and induce the activation of reactive astrocytes.

Kawarabayashi et al. (2001) indicates that the amount of detergent insoluble Aβ shows a rapid increase in Tg2576 transgenic mice starting at around 6 months of age. These levels of insoluble Aβ in the brain continue to increase rapidly from 6-10 months, eventually leading to the formation of Aβ plaques by 9-12 months of age. Detergent soluble Aβ is present throughout life.

In summary, the current literature indicates that mature Aβ plaques begin to form in the cortex and hippocampus of Tg2576 as early 9-12 months of age. Aβ deposition within plaques appears to cause the activation of astrocytes and microglia as well as the

concomitant release of inflammatory cytokines. While the direct role of Aβ in initiating hippocampal cell death is still a point of contention, it appears that the rise in insoluble levels of Aβ by 6-7 months is certainly implicated in the onset of several potentially destructive changes in the brains of these transgenic mice, such as free radical formation and initiation of the inflammatory response.

APPsw Model: Behavioral Findings

In the first study to assess the extent to which mice transgenic with the APPsw mutation exhibit a loss of behavioral function, Hsaio et al. (1996) tested Tg+ and Tg- mice in the Y-maze at 3 and 10 months of age, and the Morris Water Maze at 2, 6, and 9-10 months of age. The authors found that the Tg+ mice showed no behavioral deficits compared to their Tg- counterparts up to 10 months of age. At 10 months, however, the Tg+ mice were found to be impaired in their ability to spontaneously alternate between the three arm choices in the Y-maze. Age-matched control mice demonstrated a significantly greater percent alternation at the 10-month time point than their Tg+ counterparts. Arguably, however, Tg+ mice were already performing at chance levels at 3 months and, in that context, were impaired early in this task. 9-10 month-old mice also showed impairment, compared to age-matched controls, in their ability to locate a submerged platform in the Morris Water Maze task. The Tg+ mice required a significantly greater amount of time to locate the hidden platform on each of the 6 days of acquisition at 9-10 months, but not at earlier time points. With the hidden platform removed following the 6-day spatial acquisition period, the mice were returned to the pool, wherein their swim path was observed and recorded. It was determined that 9-10

month-old Tg+ mice spent significantly less time in the target quadrant (which previously held the platform) than their Tg- counterparts. However, Tg+ mice at 9-10 months did not spend significantly less time in the target quadrant than younger Tg+ mice at 3 and 6 months. Nonetheless, these 9-10 month-old Tg+ mice made far fewer crossings over the prior location of the hidden platform than their non-transgenic littermates. Some Tg2576 mice were later re-tested in the water maze, with spatial cues rearranged, at 12-15 months of age. Once again, the Tg+ group continued to show significant impairment in both escape latency and time spent in the goal quadrant when compared to Tg- mice, although none of this data at 12-15 months was presented. As discussed previously, Hsaio et al. (1996) found that these Tg2576 mice undergo a rapid increase in levels of $A\beta_{40}$ and $A\beta_{42}$ between 2-8 months and 11-13 months of age. Taken together, it appears that the behavioral deficits demonstrated by these Tg2576 mice could be related to the increase in brain Aβ levels/deposition.

A study conducted by Holcomb et al. (1998) focused on the differences between APPsw single transgenic mice and APPsw/PS-1 doubly transgenic mice in behavior and pathology. When Aβ load was compared between these two transgenic lines, the authors discovered that the doubly transgenic mice experienced a significant increase ($p<0.001$) in $A\beta_{42}$ and $A\beta_{40}$ from 6 weeks of age to 24-32 weeks of age while the APPsw mice showed no significant change in the amount of either Aβ species through the same time period. Consequently, by 24-32 weeks of age, the APPsw/PS-1 mice have a significantly greater level ($p<0.001$) of $A\beta_{40}$ and $A\beta_{42}$ than age-matched APPsw mice. This increase in $A\beta_{42}$ for the APPsw/PS-1 mice began before the mice reached 12 weeks of age and was followed by a second, more substantial increase in Aβ load between 12-16 weeks and 24-

32 weeks of age. Testing in the Y-maze indicated that APPsw single transgenic mice and APPsw/PS-1 doubly transgenic mice showed similarly lower percent alternations at 12-14 weeks than either the PS-1 single transgenic group or the Tg- control group. These findings seem to indicate that the APPsw mutation, and not the PS-1 mutation, is primarily responsible for generating the Y-maze impairment. Also, it appears that this impairment is unrelated to the formation of Aβ plaques, since the impairment at 12-14 weeks in APPsw mice occurs long before the onset of Aβ deposition in those mice. When these same mice were compared for the number of arm entries made, APPsw/PS-1 mice were found to have a significantly greater number of entries than any other transgenic group. APPsw and PS-1 transgenic mice were not statistically different from Tg- mice in their number of arm entries. Thus, it appears that the effect of the PS-1 mutation in AD pathology is to increase the amount of $A\beta_{42}$ in the Alzheimer's brain, inducing increased exploratory behavior/activity prior to the formation of Aβ plaques.

In a follow-up to this study (Holcomb et al., 1999), the authors re-tested all four genotypes of mice in the Y-maze at 6 months and 9 months of age. At the 6-month time point, the APPsw/PS-1 and APPsw mice were each found to be impaired in percent alternation compared to the PS-1 and Tg- groups. Only the APPsw/PS-1 mice were found to have a significantly greater number of arm entries than Tg-, although the APPsw groups showed a slightly elevated number of arm entries compared to Tg-. At 9 months, only the APPsw/PS-1 group of mice demonstrated any impairment in the Y-maze for either arm entries or percent alternation. These impairments are not progressive in nature, however, since the APPsw and APP/PS-1 groups were already performing near chance levels at 3 months of age. When tested in the Morris Water Maze at 6 or 9 months of age,

no impairments were found in any of the transgenic mice for either acquisition or memory retention. It is important to note, however, that the Tg- mice performed near chance levels at 9 months and actually had a lower percent time in the platform quadrant than the APPsw and APPsw/PS-1 groups. The authors failed to address this perplexing finding. The conclusions from this study are similar to those reached in the previous paper: the APPsw mutation is primarily responsible for the impairment in Y-maze alternation while PS-1 serves to slightly enhance AD pathology (perhaps seen here through the increase in the number of arm entries) by increasing the production of $A\beta_{42}$. None of the Y-maze behavioral changes seem to be related to Aβ deposition, as they pre-date the onset of plaque formation.

A study conducted by Westerman et al. (2002) creates a correlational link between the onset of spatial acquisition deficits and the appearance of insoluble Aβ. In the study, the authors tested four cohorts of animals bearing the APPsw mutation. These animals were tested in the Morris Water Maze at various time points from 4 months of age to 25 months of age. Each animal received a total of 36 acquisition trials, with a probe trial performed after each set of 12 acquisition trials to assess the amount of learning. Animals with age-independent deficits were eliminated from analysis. The results indicate that, prior to 6 months of age, the Tg2576 mice show no impairments in water maze escape latency or probe trial when compared to their age-matched controls. Tg2576 mice tested at 6-11 months, however, demonstrated lower mean probe trial scores, indicating that they spent less time, on average, in the goal quadrant during memory retention trials than their Tg- counterparts. Likewise, 12-18 month-old Tg+ and 20-25 month-old Tg+ mice showed significantly lower MPS than their age-matched Tg- littermates. Tg- mice at all

ages succeeded in scoring higher than chance in memory retention, such that the oldest Tg- group was statistically not different from the youngest Tg- group. In the prior acquisition phase of this task, 6-11 month-old Tg+ mice were normal compared to control. However, 12-18 month-old Tg+ and 20-25 month-old Tg+ mice were impaired in the last third of acquisition when compared to Tg-, demonstrating a significantly greater latency to locate the hidden platform. Overall, none of the Tg+ mice were impaired in acquisition when compared to their age-matched controls. These results were even more meaningful when coupled with the fact that $A\beta_{insol}$ begins to appear in the brains of Tg2576 mice at approximately 6 months of age. Less than 10% of the Tg2576 mice tested from the 4-5 month-old group contained $A\beta_{insol}$ while 70% of the 6 month-olds and 100% of the 10 month-olds possessed $A\beta_{insol}$. Detergent-soluble Aβ was present in all the age groups of Tg2576 mice tested. These results seem to indicate, therefore, that $A\beta_{sol}$ has little to no effect in the impairment of spatial memory while $A\beta_{insol}$ plays a much larger role-perhaps through pathogenic Aβ oligomeric assemblies.

Westerman et al., (2002) also demonstrated that transgenic mice possessing both the APPsw mutation and a mutant form of the PS1 mutation (APPsw/PS1) have a more accelerated conversion of $A\beta_{sol}$ to $A\beta_{insol.}$ As a result, these "bigenic" mice begin to show insoluble amyloid plaques by 3 months of age. Because these mice contain $A\beta_{insol}$ at an earlier age than APPsw, APPsw/PS1 mutants showed more impaired performance in the first probe trial than APPsw or PS1 single transgenic mice. Performance in the second and third probe trials for the APPsw/PS1 mice was not significantly different than the performance of the APPsw mice because of the steep learning curve associated with the particular strain of mice used to breed these transgenes (FVB x 129). In summary, it

appears that small Aβ oligomers are mostly responsible for the disruption in water maze spatial memory retention of Tg2576 mice (Westerman et al., 2002).

In addition to the APPsw mutation causing cognitive deficits, a study by Chapman et al. (1999) illustrates that the APPsw mutation results in a loss of long-term potentiation (LTP) in the hippocampus of affected mice. The authors tested this theory *in vitro* by inducing LTPs in the dentate gyrus and CA1 region of the hippocampus in both Tg+ and Tg- mice aged 2-8 months and 15-17 months. LTP induction was accomplished through theta-burst stimulation to the Schaffer collaterals and the perforant pathway. For the 15-17 month-old mice, the authors found that stimulus response in the CA1 and dentate gyrus were significantly higher than baseline for the Tg- mice following tetanus but the Tg+ mice demonstrated no significant increase in stimulus response. Mice in the 2-8 month-old group showed no impairments to LTP induction as both the Tg+ and Tg- mice demonstrated increased response to stimulus following tetanus. Short-term facilitation seemed unaffected in Tg+ mice, as response to the second stimulus in each pulse pair was significantly higher than the first in all groups of mice tested. Tests performed on *in vivo* mice confirmed the findings from the *in vitro* study since LTP induction was impaired in APPsw mice at the 13-15 month time-point. When 16-17 month-old mice were observed in the T-maze forced-choice alternation task, the study revealed that old APPsw mice were significantly impaired compared to their age-matched controls in their ability to learn the task. By contrast, 2 month-old APPsw mice learned the task as quickly as their age-matched controls and quicker than the 16-17 month-old APPsw mice. Pathological observations made after the LTP measurements and T-maze task indicate that the older APPsw group of mice possessed high brain levels of Aβ, while their non-transgenic

counterparts had none. Correlational analysis showed that impaired performance in the T-maze is associated with loss of LTP induction.

In a similar study, Fitzjohn et al. (2001) tested a group of APPsw mutant mice and determined that LTP induction is not impaired with age. In the study, the authors found that *in vitro* CA1 synaptic transmission is, in fact, impaired in 12 month-old APPsw mice. Despite this impairment, however, *in vitro* LTP induction was found to be normal in the CA1 region of 12 and 18 month-old Tg+ mice using paired-pulse stimuli. Similar results were obtained when examining the induction of LTP in the dentate gyrus of 18 month-old Tg+ mice. These findings seemingly contradict the results obtained by Chapman et al. (1999). Fitzjohn et al. (2001) suggests that these disparate conclusions could be explained by variations in basal synaptic transmission, possibly due to the use of kynurenate, a fixative that has been shown to mask deficits in synaptic transmission.

A study by King and Arendash (2002a) sought to settle the disparities among the behavioral literature involving Tg2576 mice by documenting the impairments of Tg+ and Tg- mice in a full behavioral test battery at 4 distinct time points. Four groups of Tg2576 and Tg- control mice were put through a six week battery of cognitive and sensorimotor tasks starting at 3, 9, 14, and 19 months of age, respectively. The authors found that Tg+ mice were more active in the open-field task at 3 months of age than their age-matched controls. While Tg2576 mice were not more active in the Y-maze for any given time point, the Tg+ mice did demonstrate a greater number of arm entries, overall, than non-transgenic mice. These two measures indicate that Tg2576 are more active and may show less habituation to their environment. To assess sensorimotor performance, the Tg2576 mice were observed in the balance beam and string agility tasks. Data collected for the

Tg2576 mice indicate that these mice are unable to remain on the beam for as long as Tg- mice at 3, 14, and 19 months of age. In general, a progressive decline in beam performance was evident for both Tg+ and Tg- mice with increasing age. Similarly, Tg2576 mice demonstrated inferior performance in the string agility task at 14 and 19 months when compared to Tg-. Likewise, string agility declined with age in both Tg+ and Tg- mice. These measures indicate that Tg2576 mice, in particular at older ages, are far less agile and have less strength/agility than their Tg- counterparts. To elucidate any cognitive impairment in Tg2576 mice, several tasks were used. The Y-maze, in addition to indicating overall activity, can serve as a cognitive measure when percent alternation is analyzed. When compared to age-matched controls, Tg2576 mice showed a lower percent alternation (impaired performance) at 3 and 19 months of age, as well as overall. Other cognitive tasks, including circular platform, Morris water maze acquisition, and Morris water maze retention, failed to demonstrate that the Tg+ mice were in any way impaired, overall or at any specific age. The Tg2576 mice were, however, impaired "overall" in the platform recognition task, as well as at 9, 14, and 19 months of age compared to Tg-. Both groups of mice displayed a decline in performance with advancing age. In the passive avoidance task, Tg2576 mice demonstrated a delayed latency to enter the dark chamber in pre-shock testing at 14 months, perhaps indicating their lack of exploratory behavior compared to Tg-. Surprisingly, Tg+ mice also displayed a greater latency to enter the dark chamber during post-shock testing at 9 and 14 months of age, perhaps due to greater fear/anxiety compared to Tg-. There was no transgenic effect, however, in the active avoidance task. Finally, Tg2576 mice experienced a significantly lower survivorship through 19 months than Tg-. Overall, it appears that, while Tg2576

mice demonstrate significant sensorimotor deficits, they experience very little cognitive impairment through 19 months of age. The nearly significant impairment at 19 months of age for the Morris water maze acquisition indicates that cognitive impairment might begin to occur beyond this time point. Another factor to consider is that the mice used in this study were of the C57BL/6 strain, which possess a particularly high level of intelligence, possible masking any transgene-induced impairments.

In a companion study to the one just described, Arendash and King (2002) sought to determine what correlations exist between behavior performance measures in each of their tasks for the Tg2576 mice carrying the APPsw mutation and non-Tg+ mice. Determining the relationships between performances in different tasks could help us identify the common features inherent in these tasks. In addition, understanding which behavioral tests are correlated with one another could help us better assess the efficacy of therapeutics and treatments against AD in transgenic mice. When the performances of all 169 mice were analyzed together, the authors found several correlations between behavioral tasks. First, it was discovered that open-field activity and the number of Y-maze entries were highly correlated, underscoring the fact that both of these tasks assess overall activity/exploratory behavior. In addition, balance beam performance and string agility were also highly correlated, demonstrating the importance of balance and dexterity in each of these tasks. All four of these tasks can be classified as sensorimotor measures. When the authors looked at "intra-task" cognitive behavioral measures, they found that performance in the water maze acquisition trials was highly correlated with memory retention in the probe trial. Secondly, the number of errors made in the circular platform task, where the mice are asked to locate a single escape hole among 16 that will lead

them away from aversive stimuli, was found to be related to the overall escape latency. In addition, a number of "inter-task" correlations were found between measures in various cognitive tasks. For example, percent alternation in the Y-maze was found to be negatively correlated with circular platform errors and latency, indicating that those animals with better alternation ability in the Y-maze were quicker to locate the escape hole in the circular platform. Platform recognition escape latency, not surprisingly, was correlated with Morris Water Maze latency and circular platform latency, suggesting that each of these tasks requires similar cognitive traits. Performance in platform recognition was also determined to be inversely related to Y-maze alternation, indicating that the Y-maze is also cognitive in nature. Finally, there were several correlations discovered between sensorimotor tasks and cognitive tasks. For example, open field activity and Y-maze entries were found to be inversely related to circular platform latency, indicating that those animals that displayed higher activity in the open field and Y-maze were quicker at escaping from the circular platform. In addition, balance beam performance was determined to be inversely related to circular platform latency, demonstrating that the circular platform task may have certain sensorimotor components to it. Platform recognition latency, likewise, was found to have a negative correlation with balance beam performance and string agility, suggesting that the platform recognition may also contain some sensorimotor aspects. The implication of this data analysis is that performance in one behavioral task can be highly predictive of performance in another task. What's more, these predictive correlations can be influenced by genetic background and age, as many behavioral tasks were only correlated with one another when mice were divided into groups of similar age and/or transgenicity. These results will go a long way towards

better understanding how behavioral measures are related and how they can be classified based on the skills they test.

In another study, King and Arendash (2002b) attempted to correlate synaptophysin staining in the cortex and hippocampus of Tg2576 mice bearing the APPsw mutation with behavioral impairments. In the study, the authors used a specific antibody to stain for synaptophysin, a 38-kDa membrane glycoprotein that is localized to neuronal synaptic vesicles. Previous reports involving various transgenic strains of mice have been inconclusive concerning the precise relationship between synaptophysin staining, Aβ plaque formation, and aging. The current belief is that increased amyloid levels/plaque formation and the development of dystrophic neurites leads to a maintained area of synaptophysin staining. In the present study, King and Arendash (2002) found that Tg+ mice retain their high levels of synaptophyin staining through time, presumably signaling the formation of plaques, while Tg- mice begin to show a decline in staining as they get older. Consequently, by the age of 19 months, the Tg2576 mice show a significantly greater amount of synaptophysin staining than their Tg- counterparts, particularly in the deep and outer layers of the neocortex as well as dentate gyrus, outer molecular layer and polymorphic layer of the dentate gyrus in the hippocampus. This difference in staining between the two genotypes could not be explained by tissue atrophy. For behavioral analysis, the study employed 43 Tg2576 mice and Tg- controls divided into four age groups: 3, 9, 14, and 19 months. The authors found that, for all animals combined, those with greatest "cortical" synaptophysin staining demonstrated a heightened level of activity, as determined through the open field activity and Y-maze entry tasks. These animals were also observed to have difficulty in locating and swimming towards a visible

platform in the water maze. Animals that possessed increased "hippocampal" synaptophysin staining were found to have impaired performance in the balance beam task as well as deficits in spatial acquisition and retention in the Morris Water Maze task. When the 19 month-old animals were analyzed separately, high levels of cortical synaptophysin staining correlated with increased open-field activity only. Synaptophysin staining in the hippocampus of the 19 month-old mice correlated with impaired performance in the balance beam, water maze, and platform recognition tasks. In addition, the study found that, among 19 month-old animals, impaired performance in water maze acquisition correlated with decreased hippocampal thickness and several thinner hippocampal strata. These findings highlight the important interactions between AD pathology and behavior in transgenic mice. The results of this study are consistent with the hypothesis that maintained SYN-IR in brains of Tg2576 mice is associated with impaired synaptic function and, consequently, cognitive deficits.

In summary, the behavioral findings described here indicate that APP overexpression, and/or the process of Aβ deposition caused by the "Swedish" mutation to the APP gene, induces deficits in cognitive and sensorimotor function in transgenic mice. While the time course and extent of these deficits is still a point of contention, the current evidence points to a direct link between an increase in the amount of insoluble Aβ oligomers/amyloid plaques and the onset of AD-like behavioral abnormalities. The existence of this link between pathology and behavior validates the APPsw transgenic line as a valuable model for testing potential therapeutics against AD.

Other Transgenic Models of Ab Overproduction

Besides the APPsw mouse, there are other AD transgenic mouse models that demonstrate an overproduction of Aβ. Among these models is the APPsw/PS-1 mouse, often called the PSAPP model. This model possesses two mutations that enhance Aβ production—the "Swedish" mutation, which increases β-secretase activity, and the A246E PS-1 mutation, which increases γ cleavage of APP. When these two mutations are combined, the result is a significant enhancement in the amount of Aβ generated in affected neurons, as well as an earlier onset of AD-like pathology. Takeuchi et al. (2000) found that PSAPP mice begin to display Aβ deposits in the neocortex, cingulate cortex, and hippocampus as early as 3 months of age. These deposits were observed to increase in size and density, encompassing the majority of the neuropil by 12 months of age. By comparison, APPsw mice in this study did not form any plaques until 6 months of age, becoming numerous only after 12 months. On average, PSAPP mice were found to contain between 19 and 73 times more Aβ deposits than comparably aged APPsw mice. Not surprisingly, PSAPP mice are found to demonstrate behavioral impairments as early as 5 months of age. Arendash et al. (2001) discovered that PSAPP mice are more active in Y-maze as early as 5 months of age. PSAPP mice are also impaired in the balance beam task beginning at 5 months of age as well as the string agility task beginning at 15 months of age. PSAPP mice also demonstrate progressive impairments in Morris water maze acquisition and radial-arm water maze working memory between 5-7 and 15-17 months of age. A follow-up study (Gordon et al., 2001), employing the same 15-17 month-old subjects, found an inverse correlation between Aβ (especially in compact Aβ deposits) in the frontal cortex/hippocampus and radial-arm water maze memory. Inverse

correlations were also found between compact Aβ loads in the frontal cortex and the delayed retention trial of RAWM testing. These findings indicate that PSAPP mice develop cognitive deficits in association with Aβ burdens.

Another transgenic mouse model that demonstrates an over expression of Aβ is the APP^{V717F}, or PDAPP model. The PDAPP model involves a point mutation on the APP protein, near the γ-secretase cleavage site, that increases γ-secretase activity. Behaviorally, an analysis of AD pathology described in a follow-up study (Dodart et al., 2000) indicated that homozygous PDAPP mice begin to show Aβ deposits at 3-4 months of age, while only half of all heterozygotes develop plaques at this age. Early plaques were limited to the CA1 region of the hippocampus, medial cingulate cortex, and corpus callosum. By 6-7 months of age, Tg+ mice start to develop plaques in the parietal and perirhinal cortices. Finally, at 12 months of age, PDAPP mice demonstrate Aβ plaques in the frontoparietal and temporal cortices, with homozygous Tg+ mice averaging 3 to 4 times more Aβ that age-matched heterozygous mice. Behaviorally, Dodart et al. (1999) examined a set of PDAPP mice and determined that Tg+ mice begin to show impairments in Radial-Arm water maze as early as 3 months of age, with homozygous mutants demonstrating a more profound level of impairment at an early age. These homozygous PDAPP mice also possess impairments in object recognition by 6 months of age, while heterozygotes remain unimpaired through 9 months of age. All PDAPP mice were shown to display greater locomotor activity from an early age. As an aside, the authors noted that most of the PDAPP mice examined in this study suffered from hippocampal atrophy and callosal agenesis from early adulthood onward---these two conditions may further impair the cognitive performance of PDAPP transgenic mice.

Tau Transgenic Models

As discussed in the introduction, the second major hallmark of AD is the formation of intraneuronal neurofibrillary tangles (NFT's). NFT's are composed of the microtubule-associated protein tau and their formation has been implicated in neurodegenerative disorders such as progressive supranuclear palsy (PSP), corticobasal ganglionic degeneration (CBD), and frontotemporal lobe dementia of the Parkinson's type (FTDP) (Kwon, 2002). Six unique isoforms of the tau protein exist in the adult human brain, depending on how the tau mRNA is spliced from its gene, which is located on human chromosome 17. These isoforms can be identified based on the presence or absence of 29 or 58-amino acid inserts in the amino-terminal half of the tau protein and a 31-amino acid repeat region located in the carboxy-terminal half. Isoforms that contain the 31-amino acid repeat are known as 4-repeat tau. Those isoforms that lack the 31-amino acid are identified as 3-repeat tau (Kwon, 2002). NFT's form when tau becomes hyperphosphorylated and dissociates from the microtubule to form paired helices that disrupt axonal conduction and, ultimately, results in the death of the neuron (Selkoe, 2001). At present time, there are three known mutations to the tau protein that result in the production of NFT's in transgenic mice: P301L, R406W, and V337M. The P301L mutation only affects 4-repeat tau isoforms, while R406W and V337M affect all tau isoforms. Each of these mutations involves changes to the microtubule binding repeat region of the tau protein, which is thought to result in the partial loss of function of tau and a reduced level of tau binding to the microtubules. Consequently, these mutations markedly increase the tendency of tau filaments to aggregate, leading to the formation of NFT's (Kwon, 2002).

Although not many studies have been performed dealing with mice transgenic for the P301L mutation to tau, two important studies describe the pathology and motor behavior associated with this mutation. In the first study, Lewis et al. (2000) observed a group of JNPL3 mice carrying the P301L mutation. As early as 6.5 months of age, the authors found that mutant P301L mice possessed neurofibrillary tangles in the diencephalon, brain stem, cerebellar nuclei, and spinal cord. The authors also found "pre-tangles" and tau-positive processes in neurons of the cortex, hippocampus, and basal ganglia. In addition, gliosis was observed in the spinal cord, brain stem, diencephalon, and basal telencephalon of Tg+ mice, with up to a 48% loss of motor neurons in the spinal cord. At 4.5 months of age, homozygous P301L mutants displayed a lack of escape extension during tail elevation, spontaneous back paw clenching while standing, a delayed righting response, and could only hold onto a string briefly before falling. Hemizygous mice began experiencing these same behaviors at 6.5 months of age. In addition, Tg+ mice demonstrated a reduction in grooming, weight loss, fewer vocalizations, and increased eye irritation. These animals began to demonstrate hindlimb paralysis by 8-10 months of age, with morbidity within 3-4 weeks thereafter. By contrast, JN4 and JN25 mice, which do not express the P301L mutation at the same level as the JNPL3 strain, failed to show any such impairment. Tg- controls, likewise, displayed normal behavior. These findings, therefore, seem to correlate the formation of NFTs in the brain and spinal cord with neuronal loss and sensorimotor abnormalities.

A study of mice bearing the V337M mutation to tau, a slightly less potent mutation than P301L, showed that these animals also demonstrate behavioral abnormalities (Tanemura et al., 2002). V337M mice at 11 months of age were found to

have increased activity in the plus maze, spending most of their time in the open arms, as well as increased activity in the open field. The V337M did not show any impairment in the Morris Water Maze. Pathologically, these mice develop NFT's by 10-14 months within the hippocampus, as evidenced by irregularly shaped neurons, an accumulation of RNA in the neuronal cytoplasm, and a lack of normal α-tubulin (indicating a loss of microtubules). In addition, an analysis of neural activity in the hippocampi of V337M mice, following an applied stimulus at the Schaeffer collaterals, indicated that Tg+ mice generate a weaker depolarization response in the stratum pyramidale and stratum radiatum. Because the P301L mutation leads to an early neuronal death in transgenic mice, it is difficult to observe its pathology. Therefore, these pathological observations of V337M mice are of special interest. The V337M mutation, unlike the P301L mutation, does not lead to neuronal cell death in mice. Instead, V337M mutant mice develop degenerative neurons, featuring atrophy of the nucleus and cytoplasm.

Tatebayashi et al. (2002) described the pathological changes and associative memory deficits in Tg+ mice with another mutation to the tau protein, R406W. Like the V337M model presented by Tanemura et al. (2002), this R406W mutation, under the control of the CaMK-II promotor, is only expressed in areas of the forebrain, specifically the hippocampus, neocortex, olfactory bulbs, striatum, and thalamus. According to the authors, R406W mice begin to show NFT formation in these forebrain areas as early as 18 months of age. Tg- mice, accordingly, fail to demonstrate any abnormal tau pathology at this same time point. The authors also report the presence of abnormally shaped neurons in parts of the hippocampus, taking on a flame-like shape. These abnormal

neurons were found to lack microtubules, but contained thin tau filaments, an observation made previously by Lewis et al. (2000) in their study involving P301L mice.

To assess the physical impairments caused by expression of the R406W tau mutation, the authors observed a group of 16-23 month-old Tg+ mice and their Tg- littermates (at an age when Tg+ mice begin to show NFT formation) through a variety of sensorimotor and learning tasks. The authors reported no significant transgenic effect in any of the sensorimotor tasks; including acoustic startle response, accelerating rotarod, and pole tests. The study did conclude, however, that Tg+ mice are deficient in certain types of associative memory. The authors discovered that R406W mice are impaired in contextual fear conditioning when tested 15 days after training, compared to Tg- controls. Tg+ mice also showed deficiencies in cued fear conditioning when tested 48 hours after receiving training. Tg- mice demonstrated a normal cued conditioning response (Tatebayashi et al., 2002).

A second study conducted by Lewis et al. (2001) presented the pathology associated with a group of transgenic mice bearing both the P301L tau mutation and the APPsw mutation. These mice were generated by crossing a line of Tg2576 mice expressing the APPsw mutation with a group of JNPL3 mice expressing the P301L tau mutation. The authors were very curious to see if the presence of brain Aβ would have an effect on the formation of NFTs, thus more closely simulating the conditions present in human AD pathology. Lewis et al. (2001) found that the APPsw/P301L (TAPP) mice and APPsw singly transgenic mice each possessed a similar number of Aβ plaques in the amygdala, olfactory cortex, cingulated gyrus, entorhinal cortex, and hippocampus starting at 6 months of age. The P301L mutation, therefore, did not result in enhanced Aβ deposition.

The TAPP mice, like the P301L singly transgenic mice, began to display NFT formation in the spinal cord and pons as early as 3 months of age. The NFTs from the TAPP mice and the P301L mice were identified as being morphologically similar to one another. Interestingly, the authors found that older female TAPP mice, between the ages of 9-11 months, displayed up to 7 times more NFTs than the age-matched P301L female cohort, primarily in olfactory cortex, entorhinal cortex, and amygdala. In addition, these female TAPP mice displayed some NFTs in the subiculum, hippocampus, and the isocortex, areas of the brain, where NFTs are rarely found in JNPL3 mice. In subcortical areas of the brain, however, there was little difference in the concentration of NFTs between female TAPP mice and P301L mice. The authors did note, however, that areas of the brain with high concentrations of amyloid plaques failed to show a concomitant increase in tangles. These results suggest that a high Aβ environment, and not the formation of Aβ plaques, is directly responsible for the increase in the number of NFTs. Like the P301L mice discussed earlier, these TAPP mice displayed similar motor disturbances and hind limb paralysis with the same age of onset. The Tg2576 mice did not show any of these impairments. In general, it appears that an interaction between Aβ (or possibly APP) and tau is occurring in the brains of TAPP mice that is leading to an increased number of NFTs and a similar level of sensorimotor and behavioral impairments as in tau mice.

Götz et al. (2001) performed a related study to better understand the relationship between Aβ and tau. In this study, the authors injected synthetic $A\beta_{42}$ fibrils into the sensory cortex and hippocampus of 5-6 month-old P301L mutant mice. A second group of P301L mutant mice were injected with the reversed sequence of $A\beta_{42}$ to serve as a control. Pathological comparisons between the test and control groups indicated that the

mice injected with $A\beta_{42}$ had up to five times more NFTs in the amygdala than the injection control P301L mice. A time-course analysis of the injected P301L mice revealed that NFTs begin to form approximately 18 days following the injection of Aβ fibrils and continue to increase in number until 60 days post-injection. Interestingly, the authors noted that the formation of NFTs occurs remotely from the site of Aβ injection. The rationale for this observation is that NFTs form in the cell bodies of neurons that terminate at the Aβ deposition site. This theory is confirmed by the fact that the P301L mice that received the Aβ injection demonstrated an increased amount of NFTs in the amygdala, where most of the neurons whose axons terminate at the site of injection have their cell bodies. Further, it appears that the induction of NFT formation by Aβ occurs through the hyperphosphorylation of tau. Only the Aβ-injected mice showed any reactivity to two antibodies that bind specifically to tau phospho-epitopes S422 and S212/T214, respectively. This finding seems to indicate that Aβ induces a mechanism that leads to the phosphorytion of tau at these two residues and the formation of abnormal NFTs.

According to the conclusions of Götz et al. (2001) and Lewis et al. (2001), it appears that the combination of $A\beta_{42}$ and mutant tau protein leads to an increased number of neurofibrillary tangles in addition to the amyloid plaques present with the APPsw mutation alone. Currently, the only mouse model that contains these two pathological hallmarks of Alzheimer's disease is the TAPP mouse, which possesses both the APPsw mutation as well as the P301L tau mutation. The creation of this mouse model is critical, as its pathology seems to more closely approximate the characteristics of human AD than

any other mouse model currently available. As such, the TAPP mouse model represents a great potential for testing possible treatments and enhanced therapeutics against AD.

Retinal Degeneration

In the literature, it has often been reported that mice and other rodents are sometimes known to suffer from a type of retinal degeneration that leads to near complete blindness in affected individuals. This retinal degeneration is caused by an autosomal recessive defect in the β-subunit of the rod-specific cGMP-PDE gene. The defect blocks the phototransduction cascade, thus causing increased cGMP levels and ensuing induction of Ca2+ channels to remain open (Ogilvie and Speck, 2002). This mutation, often abbreviated as the rd mutation, leads to the rapid degeneration of rods within three weeks after birth, eventually leading to the loss of cones, as well. While the mechanism responsible for this retinal degeneration is not fully understood, it is thought that the process involves a type of apoptotic cell death. (Ogilvie and Speck, 2002). Recently, several studies have attempted to determine the extent to which retinal degeneration might result in behavioral impairment. Spencer et al. (1995) performed such a study on a group of aged, Sprague-Dawley rats with varying degrees of spontaneous retinal degeneration. The authors found that those rats with the most severe retinal degeneration performed significantly worse in the Morris water maze than those rats with less severe retinal degeneration. Further, when the rats were re-tested in the Morris maze with the platform placed in a new quadrant, the mice with more severe degeneration were unable to learn the location of the new escape. Rats with less severe retinal damage were able to learn the new location of the escape platform relatively quickly. In addition, severely

degenerate rats spent far less time in the goal quadrant during a probe trial than less degenerate rats. Likewise, Cook et al. (2001) determined that mouse strains carrying the rd1 retinal degeneration gene display far less anxiety than rd- mouse strains when placed in an elevated zero maze, preferring to spend more time in the open arms. In contrast, a study performed by Fuller et al., (1973) indicates that rd/rd mice placed in a spatial water T-maze are not significantly impaired in their ability to locate an escape ladder over a period of 25 days of testing. The authors concede that visual cues play a negligible role in this particular water maze, perhaps explaining why rd/rd mice fail to demonstrate an impairment. Thus, it appears that retinal degeneration can have a significant effect on the performance of mice and other rodents in behavioral tasks that require visual acuity. Consequently, special attention must be placed on determining whether mice that are to be behaviorally tested are homozygous for this mutation.

While the literature is fairly complete with regards to the behavior and pathology of the APPsw mouse model, little is known about the behavioral impairments associated with the P301L tau mutation. Even less is known about the pathology and behavioral impairments associated with the Tau+APP model of AD. Given the importance of creating the most ideal model with which to test potential treatments and therapeutics against AD, it would seem vital to have a better understanding of how the behavioral impairments of P301L or APPsw+P301L mice compare to that of the APPsw mouse. The specific aims of my research, therefore, will be the following:

- Perform a full battery of sensorimotor and cognitive tests on a group of APPsw, P301L, APPsw+P301L, and Tg- control mice, in order to compare the extent of behavioral impairments associated with each of these three transgenic lines.
- Determine the effect of the retinal degeneration gene on behavioral performance across all four genotypes.
- Elucidate any correlations between the number of tau positive neurons in the brains of P301L mice and the extent of their behavioral impairment.
- Test the ability of Discriminant Function Analysis (DFA) to distinguish the four genotypes based on behavioral performance in test battery.
- Use Factor Analysis (FA) to group behavioral measures that share common factors.

Methods

Animals

A total of 32 mice in four groups were behaviorally evaluated in this study: 7 mice carrying the "Swedish" APP double point mutations $Lys^{670} \rightarrow Asn$ and $Met^{671} \rightarrow Leu$ (APPsw), 10 mice possessing the $Pro^{301} \rightarrow Leu$ tau mutation (P301L), 4 mice expressing both the P301L and APPsw mutations, and 11 non-transgenic control mice. The mice were selected from the progeny of a cross between C57B6/SJL x SW/B6D2F mice bearing the APPsw mutation and C57BL/DBA2 mice carrying the P301L mutation. All subjects employed in the study were female. Several months prior to initiation of behavioral testing, mice were individually housed in ALAC approved cages with access to rodent chow and water under a 12-hour light/dark cycle. Animal care and use was in accordance with the Guide and Use of Laboratory Animals, National Research Council, 1996, in a program and facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International, under protocols approved by the University of South Florida Institutional Animal Care and Use Committee (No. 1609, David Morgan, PhD., Principal Investigator). Behavioral testing was performed only during the day portion of the circadian cycle.

General Protocol

At 5 months of age, animals were started in a 6-week behavioral test battery (Arendash et al., 2001) to evaluate their sensorimotor abilities, anxiety levels, and cognitive performance. A few noted tasks were repeated after the completion of the initial test battery at the ages indicated. The following tasks were evaluated in the order indicated and at, or beginning at, the age indicated:

Open field activity	5M
Balance beam	5M, 6.5M, 8.5M
String agility	5M, 6.5M
Y-maze spontaneous alternation	5M, 8.5M
Elevated plus-maze (anxiety)	5M
17-measure neurologic screen	5.5M
Morris maze (acq. & retent.)	5.5M
Circular platform escape	6M
Platform recognition	6M
Radial-arm water maze	6.5M
Visual cliff (visual acuity)	7M

Upon completion of behavioral testing, all animals were euthanized at 9 months of age, wherein they were transcardially perfused with 25mL 0.9% saline according to the procedure outlined in Gordon et al., (2002). Brains of the P301L mice were promptly removed and the left hemispheres were immersion-fixed in freshly depolymerized 4% paraformaldehyde (pH 7.4) for 24 hours. The hemispheres were then cryoprotected using a series of sucrose solutions and later frozen and cut through the horizontal plane into 25µm sections using a sliding microtome and stored at 4^{o}C in Dulbecco's phosphate-buffered saline. Tau+ neurons were stained using an anti-phosphorylated tau primary antibody, incubated at 4^{o}C for 18 hrs. A biotinylated secondary antibody was added for

120 min., followed by avidin-biotin-peroxidase complex, using the Vectastain Elite kit, for 5 minutes. The Oncor V150 color image analysis system was used to quantify the number of stained Tau+ neurons in the hippocampus, cortex, amygdala, brain stem, and whole brains of the P301L mice. For each brain region, 4 to 16 horizontal sections were stained and analyzed, spaced between 2000 and 3600μm ventral to bregma. The measurement area for each region was a rectangular video field of 850,000μm^2. For each region, the measurement area was carefully positioned such that the sections from each mouse could be matched as closely as possible.

Genotyping for the rd gene, as well as the APP and Tau transgenes, was performed using PCR of a DNA sample extracted from the tails of all experimental subjects (Gordon, personal communication).

Specific Behavioral Testing Procedures

Open Field. To assess general levels of activity and exploratory behavior, each mouse was placed in the center of a square (81 x 81cm) open black box with 28.5 cm walls and lines painted on the floor to demarcate 16 squares (each 20 x 20cm). Each mouse was allowed to roam freely for five minutes inside the box. The total number of lines crossed during the 5-minute period was recorded.

Balance Beam. In order to evaluate balance and general motor function, each mouse was tested on a 1.1-cm wide beam, suspended 45.7 cm above a padded surface and supported by two columns, 50.8 cm apart. At either end of the beam was an attached 14 x 10.2 cm escape platform. Each mouse was placed on the center of the beam in a perpendicular

orientation and released for a period of up to 60 seconds. The total time spent by the animal on the beam before falling (not to exceed 60 seconds) was recorded for each trial. A total of three trials were performed in succession. If the animal was successful in escaping onto the platform, a score of 60 seconds was recorded. The average score for the three trials was calculated and recorded. This task, first done at 5 months, was repeated at 6.5 months and 8.5 months of age.

String Agility. To assess forepaw grip capacity and agility, animals were placed at the center point of a tautly-suspended cotton string for a period of up to 60 seconds. The string was suspended by the same two columns used in the balance beam task, 33cm above a padded surface. Each animal was allowed to grasp the string with only its forepaws, then released for the 60 second trial. A rating system was used to quantify each animal's string agility: "0" if the animal is unable to hang onto the string for any length of time, "1" if the animal hangs by its forepaws for 60 seconds, "2" if the animal makes an attempt to pull itself up onto the string, "3" if the mouse places both forepaws and at least one hindpaw on the string, "4" if the animal places all four paws and tail around the string with some lateral movement, and "5" if the animal escapes to the support column. The string agility score for each animal was recorded. This task was initially performed at 5 months and repeated at 6.5 months.

Y-maze. As a measure of general activity and basic mnemonic function, mice were tested in a black Y-maze with three arms measuring 21 x 4 cm and surrounded by 40-cm walls. Each mouse was placed into one of the three arms facing the middle area, and allowed to

roam the maze for five minutes. The total number of arm entries and sequence of arm entries were both observed and recorded for each mouse. Alternation, expressed in the form of a percentage, was defined as the ratio of arm entries differing from the previous two entries divided by the total number of entries. Thus, if an animal made the following sequence of arm entries (3,2,1,2,3,2,1,3), the total number of alternation opportunities would be six (total entries minus two) and the percent alternation would be 67%. Y-maze performance was initially evaluated at 5 months, and then repeated at 8.5 months of age.

Elevated Plus Maze. To assess anxiety/emotionality, all animals were evaluated using a plus-shaped maze elevated 82 cm above the floor. The maze consists of four arms, each 30 x 5 cm, including two opposite "closed" arms surrounded by dark walls and two opposite "open" arms that are exposed without any walls. In the center of the maze is a 5 x 5cm common area. For the single trial given, each mouse was placed at the center of the maze facing a closed arm, and allowed to freely explore the maze for a period of five minutes. During this trial, the amount of time (in seconds) spent in the open arms was observed and recorded, as well as the number of open arm choices and closed arm choices.

17-Measure Neurological Screen. A comprehensive neurological screen, largely derived from Irwin, (1966), was employed to further determine if any of the mice exhibited sensorimotor impairments related to their genotype. All of the mice were initially observed for transfer arousal, on a scale from 0 to 8 (with 0 = no activity) when moved out of their home cage and into a novel environment. Each mouse was then assessed for

the presence of an ataxic or hypotonic gait, as well as pelvic elevation, and tail elevation on a subjective 0 to 8 scale, with 0 being normal. Each mouse was also presented with two types of stimuli to assess their level of response. A cotton Q-tip was used to gently rub the animal's eye, in order to assess the mouse's eye blink response, or corneal opacity. The Q-tip was also used to gently poke the animal on its nose to quantify the animal's withdrawal reflex, as well as its tendency to approach the static Q-tip. A scale of 0 to 8 (8 = greatest response) was used for all three of these measures. The animal was also presented with a loud "cricket" noise. A similar scale was used to quantify the extent of the mouse's response. In addition, each animal's level of anxiety and activity in response to being touched was assessed on a 0 to 8 scale. A wire mesh grid was employed to test the mouse's grip strength using its front paws. The mesh was also used as a test of vision by evaluating at what point the mouse will reach out for the grid when suspended by its tail and lowered to a grid just out of reach. Each mouse's vision and anxiety were also tested by assessing whether it would hesitate at the edge of a visual cliff. Vision and anxiety were also evaluated in the elevated platform task, where the animal is placed in the center of an elevated circular platform (21 cm in diameter) for a period of 60 seconds. The latency to first head poke over the side, as well as the number of times the mouse poked its head over the side of the platform was observed and recorded. The mouse's righting reflex was assessed by placing the animal in an empty cage and gently shaking it while observing if the mouse is able to remain upright. This observation was recorded as a simple yes or no answer. Finally, the toe pinch and tail pinch tasks were administered to test the animal's reflexes to painful stimuli.

Morris Water Maze. To measure reference learning (acquisition) and memory (retention), mice were placed into 100-cm circular pool filled with water at 22-27^{o}C. The pool was divided into four equal-sized quadrants with the use of two black lines drawn along the bottom of the pool. The pool was surrounded by various visual cues, including a halogen lamp, beach ball, colored wall poster, camera stand, as well as the experimenter, which helped the mice to orient their location in the pool. A clear, 9-cm platform was placed in the middle of quadrant II (QII), such that the surface of the platform was exactly 1.5 cm below the water surface and visually indiscernible to the mice. Reference learning (acquisition) occurred over a nine-day period with four successive trials per day. On each trial, the animal was started from a different quadrant, with the same quadrant start pattern taking place on each day of testing. The latency to find the platform (up to 60 seconds) for each trial was recorded and a daily average recorded for each animal. After locating the platform, the mouse was allowed to remain on the platform for 30 seconds. If the animal failed to discover the location of the platform in 60 seconds, it was guided to the platform and then allowed to stay for 30 seconds. A latency of 60 seconds would be entered into the record for such an occurrence. On days 4, 7, and 10 (prior to acquisition testing, for days 4 and 7), each mouse was administered a memory retention (probe) trial by allowing the animal to swim in the pool for 60 seconds after the platform had been removed. Only one trial was performed and the mouse was allowed to start swimming from a location just opposite to the former platform quadrant. Each mouse's retention trial was recorded on videotape and the amount of time spent in each quadrant as well as average swim speed were later

calculated and recorded. Mice were always allowed to dry under heat lamps before being returned to their respective home cages.

Circular Platform. As a test of spatial learning/memory, each animal was observed in an enclosed, 69-cm circular platform with 16 holes spaced 1.3 cm apart around the periphery of the platform. A black curtain decorated with visual cues enclosed the platform. To provide aversive stimuli that would motivate the test animals to escape the platform surface, two 150-watt flood lamps were hung 76 cm above the surface of the platform and a high-speed fan was mounted 15 cm above the platform surface Escape is only possible through one of the 16 holes, where a box filled with bedding is placed underneath the hole. The escape hole was changed for each animal, such that the same animal would experience the same escape location for each day of testing, but different mice would have different escape locations. Prior to actual testing, each mouse underwent several "shaping" trials, wherein they were guided from the center of the platform to their correct escape location. Following the shaping trials, animals were tested for 8 days, with one trial per day. On each day, mice were placed in the center of the platform, facing away from their respective escape hole, and allowed five minutes to freely explore the platform. The total number of errors, defined as the number of times the mouse poked its head through a non-escape hole, and the latency to find the escape hole (up to 300 seconds) were recorded for each mouse.

Platform Recognition. To test for ability to recognize/locate a variably-placed visible platform, each mouse was tested in the same pool used for the Morris Water Maze task.

Instead of using a submerged, clear platform, however, the platform recognition task employed a 9-cm circular platform raised 0.8 cm above the surface of the water with a large 10 x 40 cm black and white ensign attached to the surface of the platform. The same visual cues used in Morris Water Maze were again used in this task. Unlike the Morris Water Maze task, however, all mice were started from the same location in the pool while the platform was moved to a different one of the four quadrants for each trial. Each mouse was tested for four days, with four trials per day. All four daily trials were averaged for statistical analysis. For each trial, mice were allowed to swim freely for 60 seconds or until they located and ascended the platform. Upon reaching the platform, the mice were given a 30 second stay. Mice who failed to reach the platform in the allotted 60 seconds were guided there and allowed to stay for 30 seconds. Following daily testing, mice were allowed to dry under heat lamps before being returned to their respective home cages.

Radial-Arm Water Maze. For assessment of working memory, each mouse was tested in the same 100-cm inflatable pool used in the Morris maze and platform recognition tasks, but with an aluminum frame insert added. This created six swim arms (30.5 cm length x 19 cm width), radially distributed around the pool from a central circular swim area of 40 cm in diameter. The aluminum walls stood approximately 5 cm above the water surface. The same visual cues used in the Morris maze and platform recognition tasks were again employed in this task. Each mouse was assessed in four successive acquisition trials and one retention trial for each of the 12 days of testing. The last of the four consecutive acquisition trials (T4) and the retention trial (T5) are an index of

working memory. On each testing day, the same clear, submerged platform used for the Morris water maze was placed at the end of one of the six swim arms. The platform location was changed to different arms for each of the 12 days of testing in a semi-random pattern. The start arms for each of the four acquisition trials (T1-T4) and retention trial (T5) were selected in a semi-random sequence from the remaining five swim arms. For each acquisition trial, the mouse was placed at the end of the selected start arm, facing the center of the pool. Each trial lasted one minute in duration, during which the mouse was allowed to leave the start arm in search of the submerged escape platform. An error was defined as each time the mouse swam into a non-goal arm or any time the mouse swam into the goal arm without successfully locating the platform. Following each recorded error, the mouse was returned (across the surface of the water) to the appropriate start arm to continue the trial. Additionally, any animal that failed to make an arm choice within 20 seconds of leaving the start arm was pulled back to the appropriate start arm, assessed an error, and allowed to continue the trial. Each trial was continued until the mouse located the platform or for a maximum of 60 seconds. Any animal that failed to locate the platform in 60 seconds and made fewer than 3 choices was assigned 3 errors for the purpose of statistical analysis. Upon reaching the platform, the mouse was allowed to stay for 30 seconds before starting the next trial. If the mouse was unsuccessful in locating the platform in 60 seconds, it was guided to the platform and then allowed to stay for 30 seconds. For each of the four acquisition trials, the number of error choices made prior to escape as well as the latency to locate the hidden platform, were each recorded. Following the final acquisition trial (T4), the mouse were allowed to dry under a heat lamp and then returned to its respective home cage for a 30-minute delay

period. After 30 minutes, the mouse was returned to the pool for a single delayed retention trial (T5). The same procedures as in the acquisition trials were followed and the mouse was once again allowed to dry before being returned to its home cage.

Statistical Analysis

On the basis of genotypic analysis discussed earlier, the 32 test subjects were divided into groups based on their transgene genotype (NT, APPsw, tau, or TAPP) as well as their rd status (+/+, rd/+, or rd/rd). Table 1 indicates the number of test subjects in each of these categories. Since rd/+ mice fail to show retinal degeneration, they were grouped with +/+ mice for the purposes of statistical analysis. To determine the relative effects of the rd gene and transgenicity on performance in each of the previously described tasks, a two-way analysis of variance (ANOVA) was performed for each task, using the *Statistica* analytical software package, with rd status and transgenicity as the grouping variables. Following the ANOVA, *post-hoc* differences between groups (pair-by-pair differences) were resolved using Fisher's LSD test.

For statistical analysis of 28 selected behavioral measures for rd status and genotypic effects, behavioral tasks were divided between those that were single day tasks and those that involved multiple days of testing. The single day tasks (e.g.: open field, balance beam, string agility, plus maze, Y-maze, and water maze retention) were analyzed using a simple two-way ANOVA with the grouping variables described above. The multi-day tasks (e.g.: Morris water maze, circular platform, platform recognition, and radial-arm water maze) were analyzed using a simple two-way ANOVA as well as a two-way repeated measures ANOVA. Unless otherwise noted, all group differences were

deemed significant at $p \leq 0.05$. For each task, animals defined as non-performers were eliminated from further statistical analysis in that task.

Table 2 indicates the relative effects of retinal degeneration and genotype, as well as the interaction between the two, on performance in each of the previously mentioned tasks. Based on recent findings describing the effects of retinal degeneration on behavioral performance (Spencer et al., 1995; Cook et al., 2001) as well as the statistical analyses described in Table 2 showing rd/rd status impairs cognitive performance in a number of tasks, it became necessary to remove all test subjects bearing the rd/rd genotype from this study. As indicated by Table 1, the removal of all test subjects bearing the rd/rd genotype left only one mouse carrying the TAPP double mutation. Since the performance of this single TAPP mouse was comparable to the performance of the APP group in all tasks except for circular platform and elevated plus maze, it was grouped with the APP subjects for all tasks except for circular platform and elevated plus maze.

After the test subject pool was reduced in number following the removal of all mice carrying the rd/rd gene, the data was re-analyzed in order to compare the performance of NT (n=7), APPsw (n=4), and tau transgenics (n=7). Group differences were calculated as described above, using transgenicity as the only grouping variable, with the following differences. String agility was analyzed using the Kruskal-Wallis non-parametric test and Mann-Whitney U-Test, instead of ANOVA. In addition to the measures previously discussed, all 19 sensorimotor battery tasks were analyzed using the Kruskal-Wallis test and Mann-Whitney U-Test, with the exception of visual cliff, head poke latency, and number of head pokes; these 3 measures were analyzed using ANOVA. Further, all single day tasks that were performed repeatedly (balance beam, Y-maze, and

water maze retention), were analyzed individually using ANOVA, then assessed with a repeated measures ANOVA to identify progressive changes between the performance of each group. In addition, an ANOVA was performed on the overall means for these tasks to assess overall performance across the time frame in question. For all multi-trial tasks (Morris water maze acquisition, circular platform, platform recognition, and RAWM), group differences on the last day of testing were analyzed using ANOVA. Finally, swim speed in the water maze retention task was determined using the *Accuroute* tracing program and group differences analyzed via ANOVA. ANOVA was also used to analyze quadrant preference within each group and percent time spent in quadrant 2 (the former goal quadrant) across all groups.

In order to group behavioral measures by their common factors, all data was evaluated through factor analysis using the *Systat* statistical software package. Factor analysis works by considering all of the collected data, irrespective of transgenicity, and grouping those measures into factors, each of which is measuring a different component of behavior (i.e.: sensorimotor function, working memory, etc.). In this way, behavioral measures related to one another might be determined, as well as how performance in one task might be predictive of performance in another task. Two separate factor analyses were run, each using a different grouping of the measures evaluated for all 18 test subjects. The first analysis employed a comprehensive 39-measure dataset, while the second analysis employed a more selective 19-measure subset of the behavioral data (see Diagram 1).

To determine whether the 3 genotypic groups (NT, APP, and Tau) could be distinguished from one another behaviorally, discriminant function analysis (DFA) was

performed on both a 32 and 15-measure dataset (see Diagram 1), using the *Systat* analytical software package. Circular platform and elevated plus maze measures were not used in DFA analyses to avoid any conflicts from grouping the single TAPP mouse with the APP group of mice in these two tasks (wherein the TAPP mouse's performance was appreciably different from that of the APP mice.) Two unique DFA analytical methods were employed in this study, direct entry method and stepwise-forward method. The direct entry method utilized all behavioral measures in the discriminant function model. In contrast, the stepwise-forward method selects measures iteratively for inclusion in the final discriminant function model based upon their variance contribution; only those measures that best discriminate between groups are included in the final model.

Diagram 1: Measures Included in Multivariate Analyses

39-Measure FA	19-Measure FA	32-Measure DFA	15-Measure DFA
OF	■	■	■
BB 1	■	■	■
BB 2		■	
BB 3		■	
BB Avg		■	
STR 1	■	■	■
STR 2		■	
STR Avg		■	
YM-Alt 1	■	■	■
YM-Ent 1	■	■	■
YM-Alt 2		■	
YM-Ent 2		■	
YM-Alt Avg		■	
YM-Ent Avg		■	
EPC			
EPO			
EPT			
WM Fin	■	■	■
WM Avg	■	■	■
WM RET 1		■	
WM RET 2		■	
WM RET 3		■	
WM RET Avg	■	■	■
CPE Fin	■		
CPE Avg	■		
CPL Fin	■		
CPL Avg	■		
PR Fin	■	■	■
PR Avg	■	■	■
RAWM B4T1E	■	■	■
RAWM B4T4E	■	■	■
RAWM B4T5E	■	■	■
RAWM T4E	■	■	■
RAWM T5E	■	■	■
RAWM B4T1L		■	
RAWM B4T4L		■	

Finally, correlation analysis was performed, using the

Systat analytical software package, to determine whether a relationship exists between the number of tau+ neurons in the brains of P301L mice and behavioral measures collected from the same animals. Five measures of tau pathology (number of tau+ neurons in the brainstem, cortex, hippocampus, amygdala, and whole brain) were used for this correlation analysis with the same 39 behavioral measures used for factor analysis (see Diagram 1)

Results

Behavioral Effects of the rd Genotype

Table 1 shows the distribution of rd genotypes, by transgenic group, for all 32 animals in the study. Significant percentages of animals in all four groups were determined to be homozygous recessive (rd/rd) for the retinal degeneration gene, with the remaining animals being heterozygous (unaffected carriers) or normal. To determine the effect of rd homozygosity and transgenicity on behavioral performance, behavioral measures from all 32 animals were analyzed for main effects of rd homozygosity and transgenicity, as well as for the interaction between the two. As summarized in Table 2, a main effect of rd homozygosity was evident in multiple behavioral measures from most cognitive-based tasks—particularly Morris water maze, platform recognition, and the RAWM tasks. These effects of rd homozygosity on cognition were always deleterious in impairing performance in tasks requiring good eyesight. By contrast, sensorimotor tasks (e.g.: open field activity, balance beam, and string agility) were not affected by rd homozygosity. Also unaffected were anxiety measures in the elevated plus maze and Y-maze spontaneous alternation, a cognitive-based task not reliant on good eyesight.

Given the wide-spread deleterious effects of rd homozygosity on performance in multiple cognitive-based tasks/measures, data from all rd/rd mice (n=14) was eliminated from the remainder of this study so that transgenicity effects could be unequivocally evaluated. In addition, the one Tau+APP mouse that was not rd homozygous was

Table 1: Distribution of wild-type (+/+), heterozygous (+/rd), and homozygous (rd/rd) mice by Genotype

Genotype	Total	+/+ or +/rd	rd/rd
Non-Transgenic	11	7	4
Tau	10	7	3
APP	7	3	4
Tau+APP	4	1	3

Table 2: Effects of rd homozygocity and genotype on behavioral performance (P values indicated)

Behavioral Measure	rd/rd effect	Genotype effect	Interaction
Open Field	0.83	0.18	0.28
Balance Beam 1	0.41	**<.01**	0.94
Balance Beam 2	0.10	**0.02**	0.87
Balance Beam 3	0.14	**0.01**	0.52
String 1	0.65	0.98	0.41
String 2	0.91	0.78	0.47
Plus Maze (#CA)	0.77	0.73	0.50
Plus Maze (#OA)	0.51	0.34	**0.04**
Plus-Maze (time in OA)	0.30	0.29	**<.01**
Y-maze 1 (Entries)	**0.02**	**0.05**	0.07
Y-maze 2 (Entries)	0.19	0.54	0.89
Y-maze 1 (%-Alt)	0.27	0.13	0.22
Y-Maze 2 (%-Alt)	0.67	**0.01**	0.35
Morris Maze Avg. Acqusition	**<.01**	0.28	0.24
Morris Maze Acq. Final Day	**0.01**	0.29	0.24
Morris Maze Avg. Probe	**0.01**	0.16	0.20
Morris Maze Probe 1	**0.05**	**0.01**	0.92
Morris Maze Probe 2	**0.05**	0.75	0.66
Morris Maze Probe 3	0.10	0.57	0.16
Circular Platform Avg. Errors	0.48	0.30	0.62
Circular Platform Avg. Lat.	0.14	0.13	**0.04**
Platform Recognition Avg.	**<.01**	0.52	0.30
Platform Recognition Final Day	**<.01**	0.22	0.66
RAWM Errors T1 Avg.	**<.01**	0.79	0.22
RAWM Errors T4 Avg.	**0.01**	**<.01**	0.58
RAWM Errors T5 Avg.	**0.04**	**0.02**	0.40
RAWM Latency T1 Avg.	**0.01**	0.28	0.53
RAWM Latency T4 Avg.	**0.01**	**0.04**	0.30
RAWM Latency T5 Avg.	**0.01**	**0.04**	0.15

#CA = # of closed arm choices #OA = # of open arm choices

Note: Significant P values (<0.05) are highlighted in bold type and box shaded

comparable in behavioral performance to animals in the APP group on most tasks (except circular platform and elevated plus maze); it was thus added to the APP group for behavioral analysis of those tasks.

Effects of APP and Tau Transgenicity on Sensorimotor and Anxiety-based Tasks

In the balance beam task (Fig. 1), both APP and Tau transgenics were impaired at 5 months, while only the APP group was impaired in balance beam performance at 6.5 months. Overall, the APP transgenic group was impaired between 5 and 8.5 months compared to Tg-, with a time x genotype interaction [$F(4,30)=2.70$; $p<0.05$]. For string agility, no significant group differences were observed at either 5 months or 6.5 months, although Tau transgenics approached impairment ($p=0.065$) at 6.5 months. Over both time points, the Tau transgenic group was nearly impaired ($p=0.07$) compared to NT mice (data not shown).

The 17-measure Neurologic Exam revealed no group differences, except that APP transgenic mice had 1) a significantly depressed pelvis during locomotion compared to both Tg- ($p<0.0001$) and Tau ($p=0.022$) groups, and 2) an abnormally hypotonic gait compared to both Tg- ($p=0.049$) and Tau ($p=0.049$) groups (data not shown).

No group differences in activity/exploratory behavior were observed, as tested in the open field at 5 months and the number of Y-maze entries at 5 months and 8.5 months (data not shown).

In elevated plus maze testing, neither transgenic group expressed higher anxiety that Tg- controls in any of the three measures analyzed (number of open arms entered, % time in open arms, and number of closed arms entered) (data not shown).

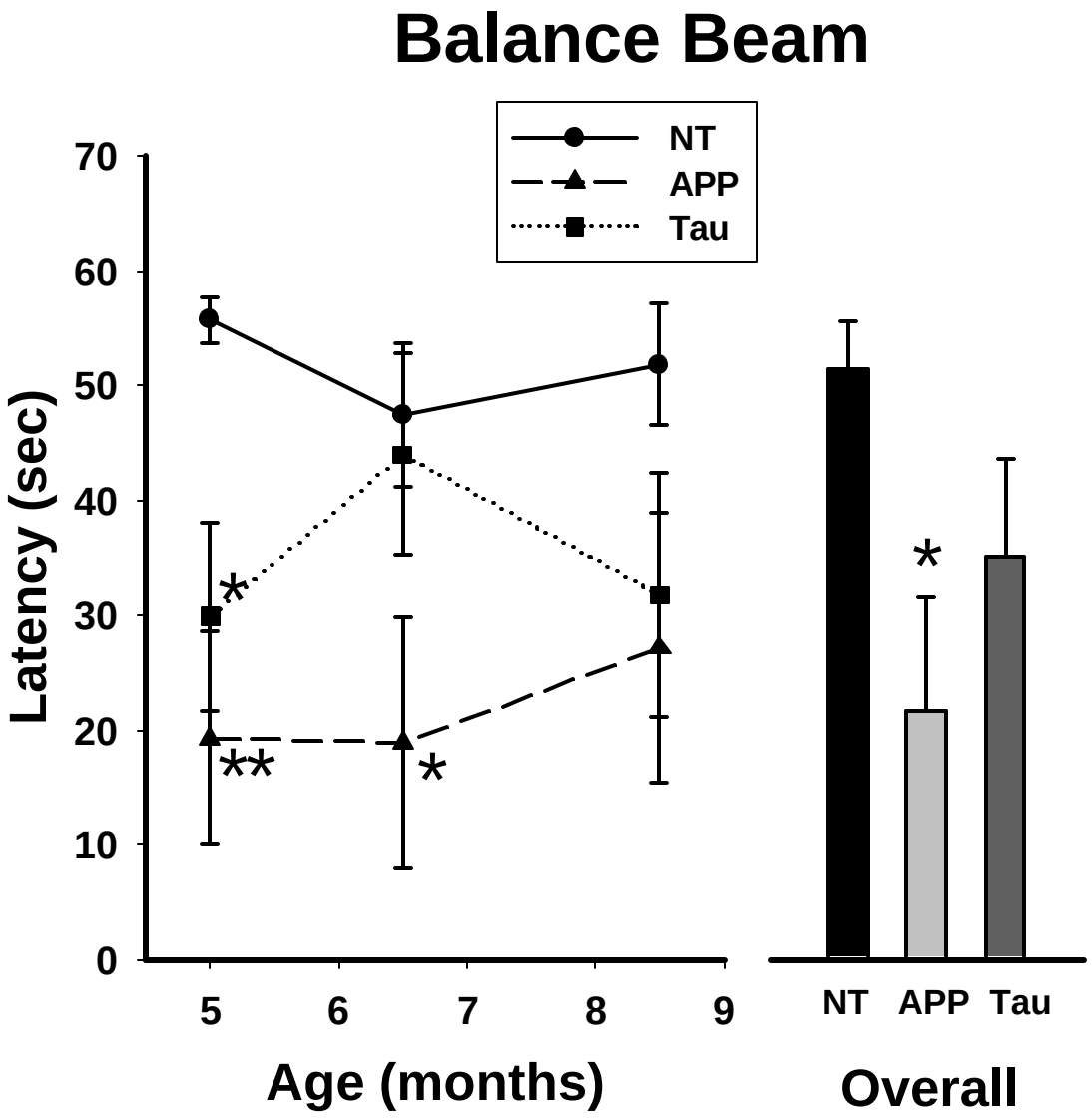

Figure 1. A comparison of balance beam performance by age and transgenicity (left) and overall (right) for APP and Tau transgenic mice and NT control mice. Balance beam performance was measured by latency to apparatus over 3 successive trials. *p<0.05 compared to NT at that age (left) or overall (right). **p<0.01 compared to NT at 5M.

Although APP and Tau mice were found to be impaired in the balance beam, performance in all other sensorimotor tasks (including those for visual acuity) and on the anxiety task (with the exception of hypotonic gait and pelvic elevation in APP mice) were normal. Thus, neither transgenic group has generalized sensorimotor dysfunction or heightened anxiety that might significantly compromise performance in the cognitive-based tasks.

Effects of APP and Tau Transgenicity on Cognitive-based Tasks

For Y-maze % spontaneous alternation, Tg- and Tau mice improved their performance between the 5-month and 8.5-month test points to about 70% alternation, while APP mice performed at chance levels (~50%) at both ages (Fig. 2). Compared to Tg- mice, APP mice were nearly impaired at 8.5 months ($p=0.07$) and significantly impaired overall vs. Tg- mice (51.3±5.1% vs. 65.4±3.3%; $p<0.05$).

In Morris maze acquisition (Fig. 3), both Tg- and Tau mice nicely reduced their escape latencies through the 9 days of testing. By contrast, APP mice showed no improvement, being significantly impaired in comparison to both Tg- and Tau mice across all 9 days of testing ($p\leq0.05$), and especially over the last block of 3 days ($p<0.007$). Even on the final day of acquisition, APP mice continued to show significantly higher latencies than Tg- mice ($p<0.02$). A genotype x day interaction was also present [$F(16,120)=2.55$; $p<0.005$].

During Morris maze probe trials for memory retention, Tau mice performed similar to Tg- mice in all three probe trials and overall (Fig. 4). However, APP mice were impaired on the final probe trial (Day 10) compared to Tg- mice ($p<0.05$). Moreover,

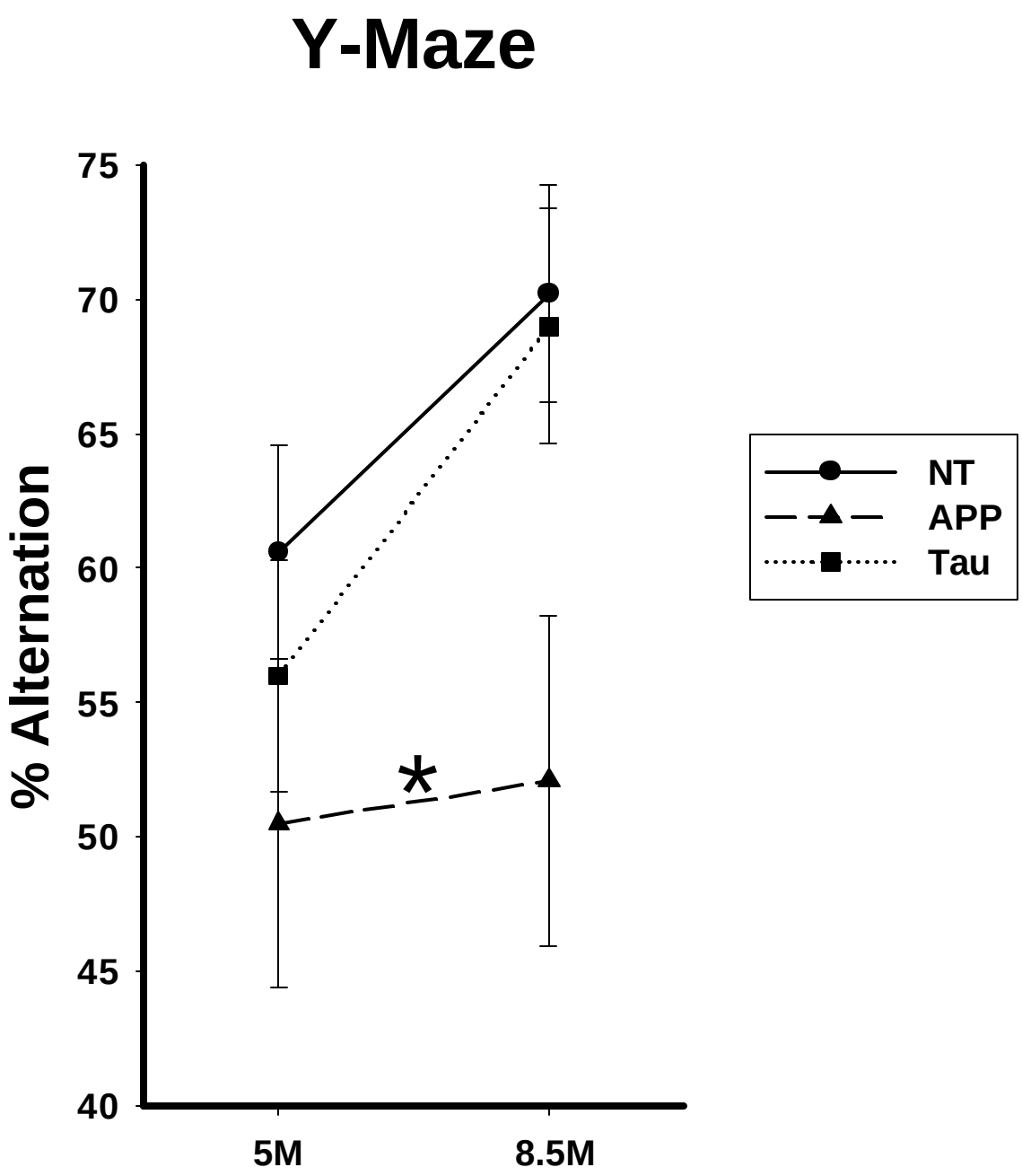

Figure 2. Y-maze percent alternation for APP and Tau transgenic mice and NT controls at 5M and 8.5M. *p<0.05 compared to NT overall.

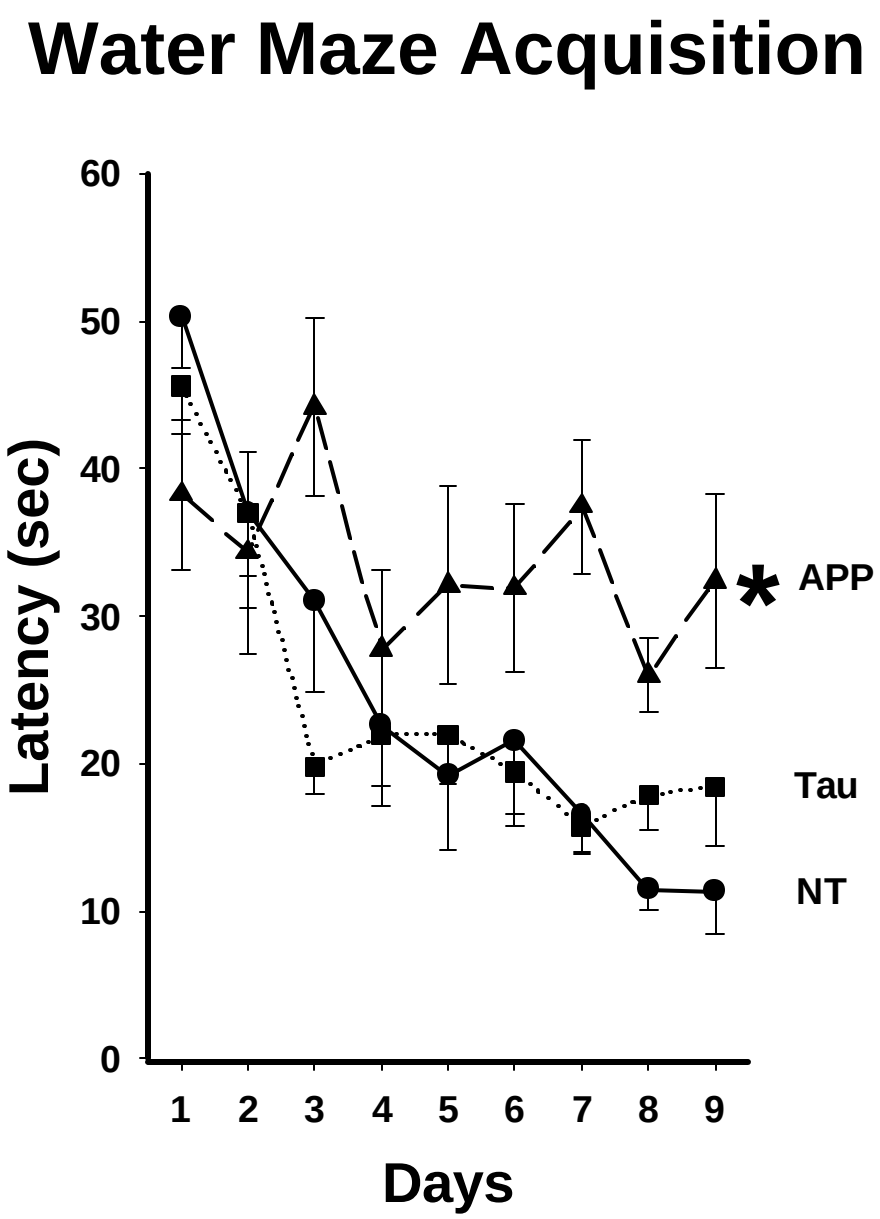

Figure 3. A comparison of Morris water maze escape latency between APP and Tau transgenic mice and NT controls across 9 days of acquisitional testing. *$p<0.05$ compared to NT and Tau mice over all 9 days of testing.

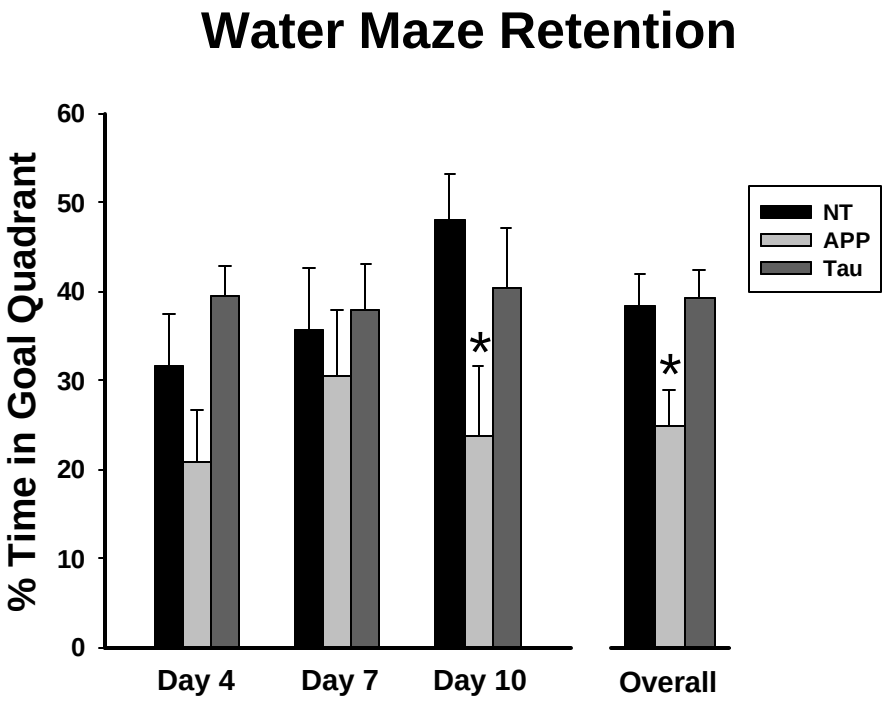

Figure 4. Morris water maze retention performance for APP and Tau transgenic mice and NT controls. Retention performance is measured as percent time spent in the goal quadrant for each of the three retention trials (left) and overall (right). *p<0.05 or compared to NT on day 10 (left) and compared to NT and Tau mice overall (right).

APP mice were impaired over all three probes in comparison to both Tg- and Tau mice ($p<0.02$; Fig. 4). There were no differences in swim speed between the three groups (data not shown).Over the eight days of circular platform testing, no significant genotype effect was evident, with all three groups making a similar number of errors and having similar escape latencies (data not shown).

Across the four days of platform recognition testing, Tau mice had escape latencies comparable to Tg- controls (Fig. 5). By contrast, APP mice showed impaired recognition abilities by having higher escape latencies overall compared to Tg- mice ($p<0.05$); this impairment was particularly apparent during day 1 (vs. both Tg- and Tau groups; $p<0.05$) and during day 3 (vs. Tg-; $p<0.01$). Nevertheless, none of the three groups differed significantly on the final day of platform recognition testing.

Figure 6 presents the number of errors in RAWM working memory testing across four 3-day blocks for Trial 1 (randomized initial trial), Trial 4 (final acquisition trial), and Trial 5 (delayed retention trial). Tau mice performed similar to Tg- mice, although they made almost significantly more T4 errors vs. Tg- mice during the critical last block of testing ($p=0.06$). Compared to both Tg- controls and Tau transgenic mice, APP mice made significantly more working memory errors in Trial 4 of block 2, as well as in Trial 5 during the last three blocks of testing. The T5 impairment of APP mice was so prevalent that a strong genotype x blocks interaction was present [$F(6,42)=3.70$; $p<0.005$].

The impaired RAWM performance of APP mice is further underscored when errors are averaged over all four blocks of testing (Fig. 7). APP mice made significantly more T4 errors overall than Tg- controls ($p<0.02$) and more T5 errors overall than both

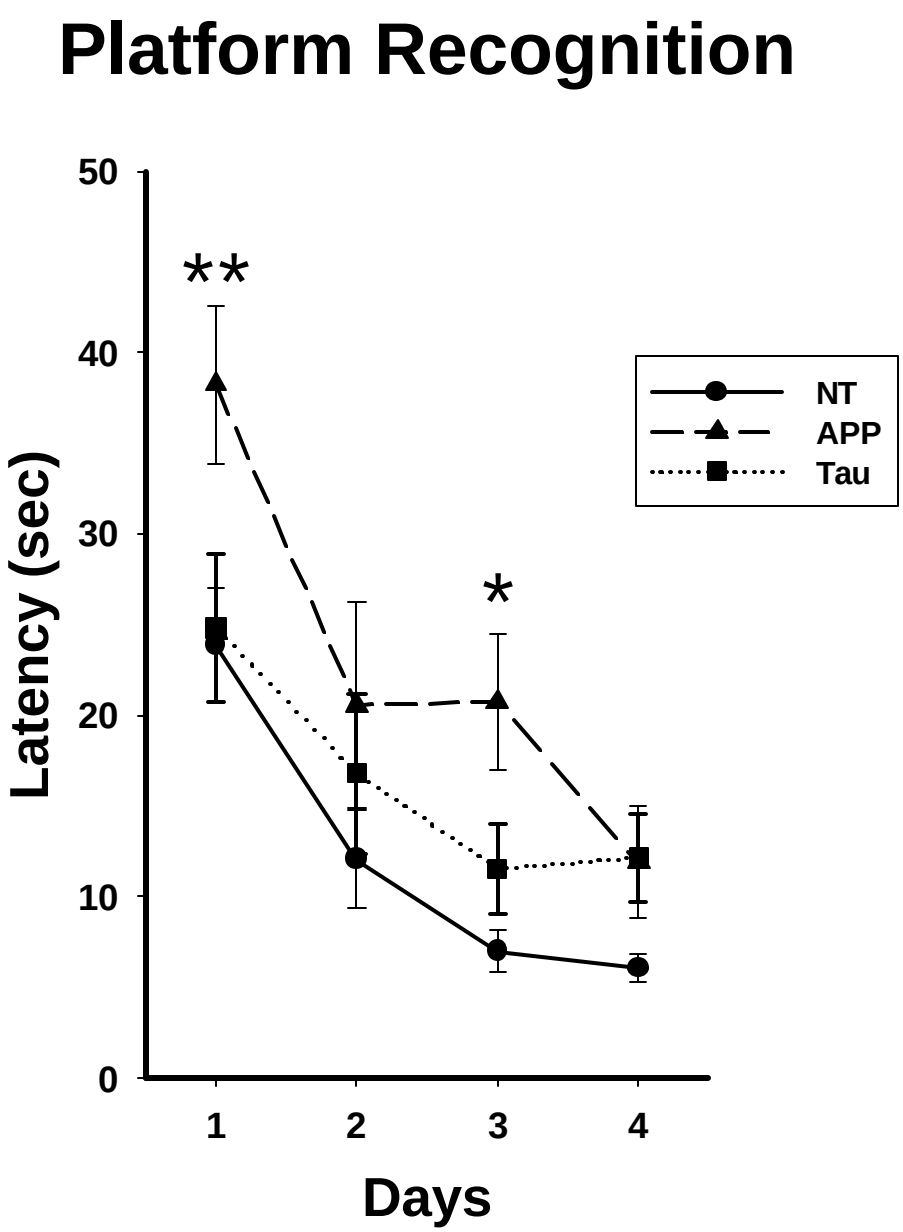

Figure 5. A comparision of platform recognition latency between APP andTau transgenic mice and NT controls across four days of testing. ** $p<0.05$ or higher level of significance compared to NT and Tau on day 1 of testing. * $p<0.05$ or higher level of significance compared to NT on day 3 of testing.

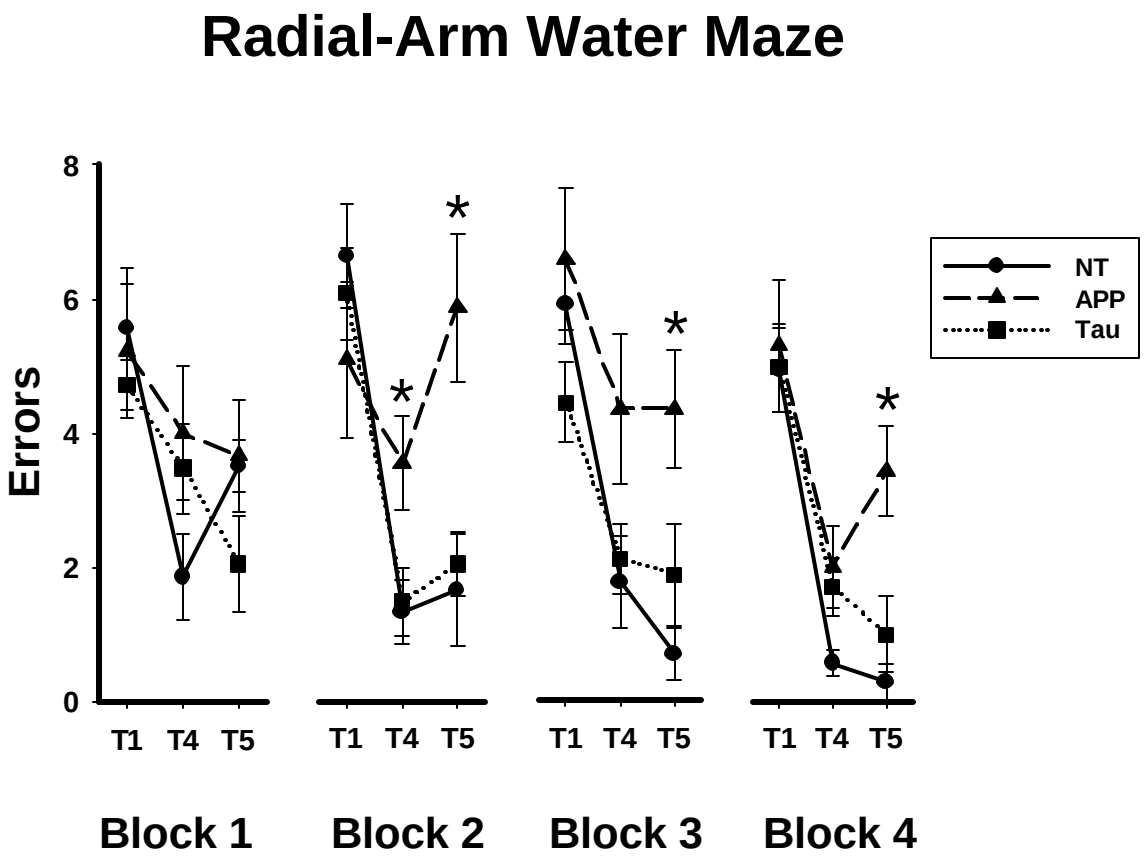

Figure 6. Radial-arm water maze errors for APP and Tau transgenic mice and NT control mice across four 3-day blocks of testing for T1, T4, and T5. *p<0.05 or higher level of significance compared to both NT and Tau mice for that particular block and trial.

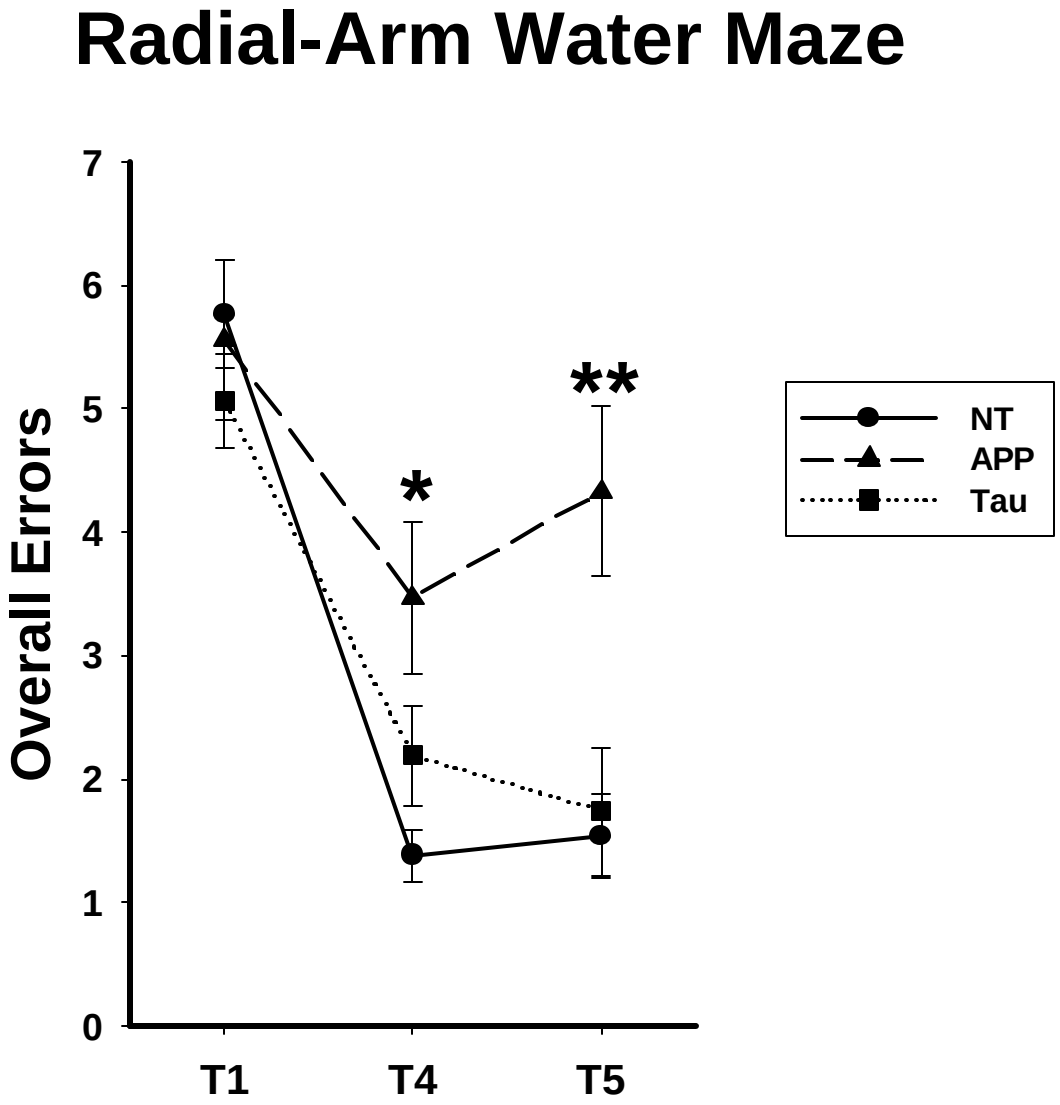

Figure 7. A comparison of radial-arm water maze errors for T1, T4, and T5 in APP and Tau transgenic mice and NT controls across 12 days of testing. *p<0.05 compared to NT controls. **p<0.01 compared to NT and Tau mice.

Tg- and Tau mice ($p<0.01$). APP mice were unable to improve their overall working memory performance from T1 through T5 (Fig. 7), whereas both Tg- and Tau mice were able to substantially reduce their number of errors over the same overall trials. Statistical evaluation of RAWM latency measures revealed very similar results to those obtained by analysis of RAWM errors (data not shown.)

Factor Analysis

Factor analysis of behavioral measures was performed to determine the underlying relationships between tasks. Table 3 indicates the task/measure loadings when all 39 behavioral measures were included in the analysis. Five principle factors were obtained. Measures for RAWM, platform recognition, and Morris maze acquisition loaded heavily under Factor 1, which accounted for more variance (25.2%) than any other factor. This factor encompassed multiple cognitive domains, including working memory, recognition, and reference learning. Morris maze memory retention loaded independently on Factor 5, indicating an independent loading of reference memory separate from other cognitive domains. Measures from two other cognitive-based tasks, circular platform and Y-maze spontaneous alternation, also loaded on factors separate from Factor 1. Factor 2 loaded balance beam and string agility measures, indicating this factor's sensorimotor basis, specifically involving balance/agility.

Similar factor loadings were obtained when a subset of 19 measures was used in the factor analysis (Table 3). The 19-measure subset (which omits all intermediate measures, redundant RAWM latency measures, and elevated plus maze measures) has been used routinely in our prior studies (Leighty et al., 2002). For the six primary factors

Table 3: Task loadings resulting from 39- and 19- measure factor analysis involving all rd- animals (n=18)

Factor	39 Measures	19 Measures
I	**(25.2)** RAWM working memory Platform Recognition Morris Maze acquisition	**(25.0)** RAWM working memory Platform Recognition Morris Maze acquisition
II	**(16.1)** Balance Beam String Agility	**(16.4)** Circular platform (errors and latency)
III	**(13.8)** Y-maze (% Alt. and entries) Circular platform (errors)	**(15.8)** Open Field activity
IV	**(10.1)** Circular platform (latency) Elevated Plus-Maze (O.A.)	**(9.5)** Balance Beam
V	**(7.6)** Morris Maze retention	**(7.9)** String Agility
VI	---	**(6.8)** Morris Maze retention

O.A. = # of open arm entries and time spent in open arms

Numbers in parentheses are percent of total variance explained

obtained, Factor 1 was again heavily based on cognitive domains provided by measures in the RAWM, platform recognition, and Morris maze tasks. Also consistent with the 39-measure analysis, Morris maze reference memory and circular platform measures loaded independently and separate from the primary cognitive factor (Factor 1). However, balance and agility measures had separate loadings for the 19-measure data set.

Discriminant Function Analysis (DFA)

To determine whether the behavioral performance of the three groups (NT, Tau, and APP) could be used to distinguished them from one another, Discriminant Function Analysis (DFA) was performed (Table 4). The same behavioral measures were included as for Factor Analysis, except that the four circular platform and three elevated plus maze measures were omitted.* For the resulting 32- and 15-measure data sets, the direct entry DFA method (which includes all measures) could not discriminate between the three groups based on their behavior. In sharp contrast, the step-wise forward DFA method (which selects measures based on their contribution to variance) was highly effective in completely discriminating between all three groups using either 32- or 15-measure data sets (Table 4). For both step-wise forward DFA analyses, 8 measures provided maximal discriminablilty. These distinguishing measures were mostly cognitive-based, being taken from RAWM, Morris maze, and platform recognition tasks. Thus, these tasks are particularly important, and sufficient, to behaviorally distinguish between all three groups.

Table 4: 15- and 32-measure discriminant function analysis of Tg-, APP, and Tau groups

Measures	Direct Entry Method (All measures used)	Step-wise Forward Method	
		Significance	Measures Used
15	**Not Discriminable**	p<.0001*	RAWM B4T4 errors RAWM T5 errors (average) Morris Maze acquisition (Final Day) Morris Maze retention (average) Platform Recognition (Final Day) Platform Recognition (average) Balance Beam String Agility (average)
32	**Not Discriminable**	p<.0005*	RAWM B4T4 errors RAWM T5 latency (average) Morris Maze retention (Day 7) Platform Recognition (Final Day) Platform Recognition (average) Balance Beam String Agility (average) Open Field

*Significant p values are for Wilks' lambda, designating overall discrimination between the 3 groups (Tg-, APP, and Tau). Post-hoc pair-wise comparisons were all significant at $p \leq 0.05$ or greater level of significance, thus providing complete discrimination between the two groups.

*These two tasks were omitted because behavioral scores of the single APP+Tau mouse included in the APP group were not consistent with other APP animals in the these two tasks. Omission of this animal's data from these two tasks would have eliminated all of that animal's data from the other tasks for DFA. As well, neither task showed genotypic differences in performance.

Correlations Between Cognitive Performance and Tau Pathology

For the Tau transgenic group alone (n=7), a number of significant correlations were found between cognitive performance in the three water-based tasks and the number of Tau+ neurons in the neocortex and hippocampus (Table 5). Escape latencies during the final day of Morris maze acquisition and in both platform recognition measures were highly correlated with Tau+ neurons numbers in neocortex. Thus, poorer performance in both tasks correlated with increased number of Tau+ neurons. Moreover, RAWM T5 errors in the final block and overall T5 latency, as well as overall platform recognition latency, correlated with Tau+ neurons in hippocampus. Thus, poorer working memory (RAWM) and recognition correlated with increased numbers of Tau+ hippocampus neurons. Three examples of these correlations are depicted in Figure 8.

Table 5: Correlations between number of Tau+ neurons and cognitive performance in Tau transgenic mice (N=7) ("p" values indicated for significant correlations)

Behavioral Measure	Tau+ Neurons in Neocortex	Tau+ Neurons in Hippocampus	Tau+ Neurons in Whole Brain
Morris Maze Latency (Last day)	**0.016**	n.s.	**0.025**
Platform Recognition Latency (overall)	**0.000**	**0.013**	**0.000**
Platform Recognition Latency (Last day)	**0.007**	n.s.	**0.012**
RAWM errors (Last Block, T5)	n.s.	**0.036**	n.s.
RAWM latency (T5 average)	n.s.	**0.022**	n.s.

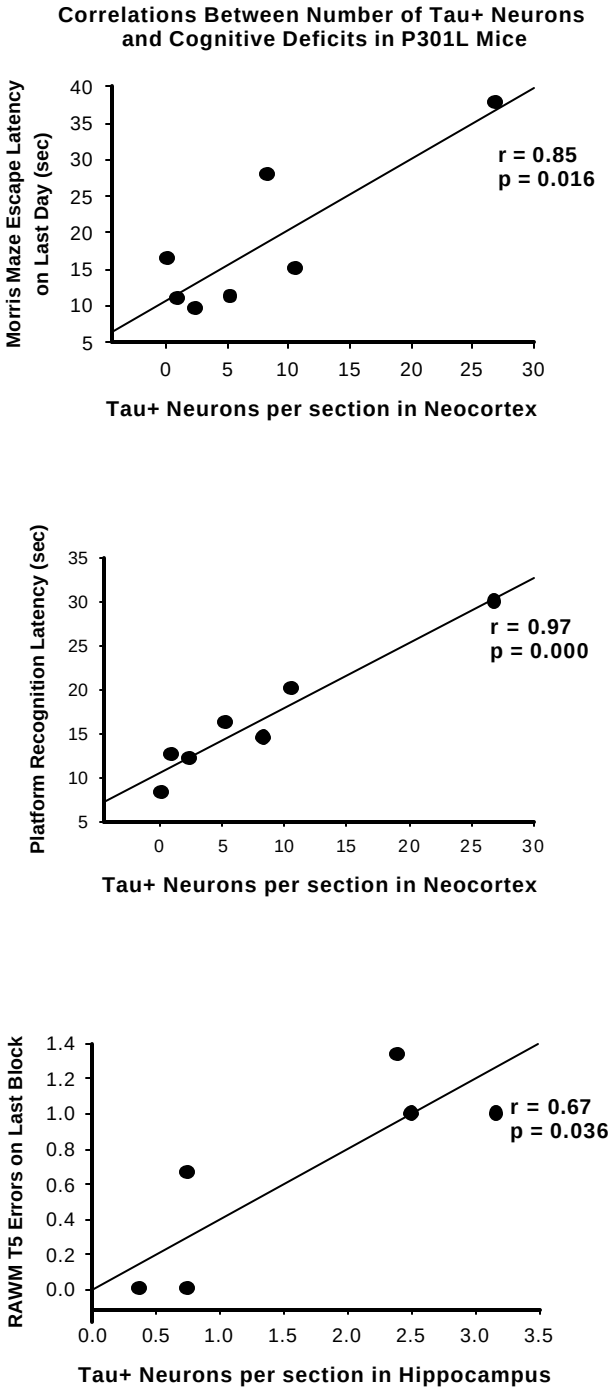

Figure 8. Graphs indicating the significant correlations between number of tau+ neurons in cerebral cortex and hippocampus of P301L mouse brains and cognitive behavior measures in the three water-based tasks. Significant r values and p values for the correlations are listed.

Discussion

General Summary

The results of this study indicate that retinal degeneration seriously impairs the performance of mice in behavioral tasks requiring good vision. Animals that carry the homozygous allele of this mutation must, therefore, be eliminated from any such study requiring visual acuity. Young APP mice were found to be impaired in several cognitive tasks, including platform recognition, Morris maze, and Y-maze, as early as 5.5 months of age. These mice were, however, found to have fairly normal sensorimotor function, demonstrating significant impairment only in balance beam performance starting at 5 months. P301L mutant tau mice were not found to possess significant impairments in any sensorimotor or cognitive tasks through 8.5 months of age. Factor analysis was successfully able to group those behavioral measures that shared the most in common and contributed the most variance to the behavioral data. Likewise, discriminant function analysis (DFA) was able to accurately discriminate between the three transgenic groups of mice using only an 8-measure data set. Finally, the use of correlation analysis demonstrated an association between the formation of NFT's in cortex and hippocampus and cognitive impairments in P301L mice.

Effects of Retinal Degeneration

Through the use of genotyping, it was determined that most of the 32 animals selected for this study were either unaffected carriers (rd/+) of the rd gene or homozygous recessive (rd/rd), predisposed to developing retinal degeneration. A significant number of animals from each transgenic line were (rd/rd) and thus suffered from retinal degeneration. To determine the extent to which retinal degeneration played a part in the behavioral impairment of these animals, all subjects' behavioral scores were assessed using both genotype and rd status as independent grouping variables. Performance in several behavioral tasks, including Morris water maze, platform recognition, and radial-arm water maze, were negatively affected by retinal degeneration. These findings illustrate that poor vision, caused in this case by the retinal degeneration (rd) gene, significantly impairs behavioral performance in those tasks that require keen vision. Performance in sensorimotor tasks, such as balance beam, string agility, and open field, which require less visual acuity, failed to demonstrate any significant correlation with retinal degeneration. Spatial acquisition tasks, such as Morris water maze, platform recognition, and RAWM, which require visual acuity, demonstrated the most significant impairments due to the rd gene. These findings agree with the results obtained by Spencer et al. (1995), indicating that retinally-degenerate aged rats, albeit not caused by a genetic mutation, had behavioral impairment in the Morris water maze (a spatial acquisition task). A study conducted by Fuller et al. (1973) found, however, that rd/rd mice showed no impairment in a spatial water T-maze that required that test subjects locate an escape ladder found in one of the two swim arms. The authors suggest that these results may be an indication that spatial cues are not a significant component of

learning in this task. These results are in agreement with our findings that the rd mutation does not significantly affect performance in the Y-maze spontaneous alternation task, a similar task that requires cognitive function with limited visual acuity. Curiously, my findings contradict the results obtained by Cook et al. (2001), who reported that rd/rd mice show less anxiety when placed in an elevated zero maze, preferring to spend more time in the open arms of the maze than the closed arms. My findings indicate no significant difference between rd/rd mice and mice with normal vision (+/+ and rd/+) in the amount of time spent in the open arms or the number of open arm entries made in the elevated plus maze, a similar task used to measure anxiety.

Because rd/rd test subjects were found to have significant impairments in various tasks, irrespective of transgenicity, all such animals were excluded from further statistical analysis. The elimination of these rd/rd mice reduced the number of experimental animals from 32 to 18: 7 Tg- mice, 7 Tau mice, 3 APP mice, and only a single Tau+APP mouse. The performance of the single TAPP mouse did not significantly differ from the performance of the remaining three APP mice in all tasks except circular platform and elevated plus maze, therefore it was grouped with the APP mice for the purposes of statistical analyses.

Effects of APP and Tau Transgenicity on Sensorimotor and Anxiety-Based Tasks

To assess levels of anxiety and sensorimotor function in transgenic and non-transgenic mice, all test subjects were evaluated in a variety of tasks. According to my findings, neither of the two transgenic groups showed differences in exploratory behavior compared to Tg-, as measured by activity in the open field task and the number of entries

made in the Y-maze spontaneous alternation task. These observations are in agreement with the findings put forth by Holcomb et al., (1999), indicating that APP mice are not significantly different from Tg- in their number of Y-maze entries at 6 and 9 months. King and Arendash (2002a) reported that APP mice are more active in the open field, compared to Tg-, at 3 months of age, but not at 9 months. My findings, which involve an intermediate time point, indicate that APP mice are not more active in the open field at 5 months compared to Tg-.

Both transgenic lines did, however, experience some impairment in the balance beam and string agility tasks. When evaluated at 5 months of age, both the APP and Tau groups had significant impairment in the balance beam when compared to Tg-, while only the APP mice exhibited similar impairments when the animals were re-tested at 6.5 months of age. Neither transgenic group showed impairments at the final time point of 8.5 months. Overall, the APP mice were significantly impaired in their balance beam performance when compared to Tg-, while the performance of the Tau mice was not significantly different from control. While neither transgenic group had statistically significant deficits in string agility, the Tau group did exhibit nearly-significant impairments in string agility at 6.5 months of age ($p=0.065$) and overall ($p=0.07$), compared to Tg-. King and Arendash (2002a) agree with my observation that APP mice are impaired in the balance beam at an early age. The authors found that APP mice are impaired in balance beam at 3, 14, and 19 months of age, but not at 9 months, while my observations indicate that APP mice are impaired at 5 and 6.5 months of age, but not at 8.5 months. In addition, King and Arendash (2002a) indicate that APPsw and Tg- mice

are not significantly different in string agility at 3 and 9 months of age, which agrees with my observation that APP mice are not impaired in string agility at 5 or 6.5 months of age.

Neither group experienced heightened anxiety as evidenced by the fact that both the APP and Tau groups spent similar amounts of time in the open arms of the elevated plus maze and made a similar number of open arm choices compared to Tg-.

Finally, the 17-Measure neurologic screen revealed that APP mice suffered from a mild hypotonic gait and slightly depressed pelvis. Neither of these conditions, however, precluded the APP mice from performing in any of the behavioral tasks.

While little is known regarding the behavioral impairments associated with mutations to the tau protein that lead to NFT formation, there are a few significant findings that relate to the observations made in this paper. Lewis et al., (2000) report that P301L mice display severe sensorimotor deficits as early as 4.5 months of age in homozygous animals and beginning at 6.5 months in hemizygotes. These deficits include the lack of an escape extension during tail elevation, spontaneous back paw clenching while standing, a delayed righting response, an inability to hang from a suspended string for more than a few seconds, and decreased grooming, weight, and mobility. While my study did not address such variables as body weight and grooming, the observations made during an extensive array of sensorimotor tasks reflect the relatively normal function of all Tau mice through the 5 to 8.5 month test period. As discussed previously, all Tau mice in this study displayed normal ambulation and activity, as well as proper sensorimotor function and neurological response to stimulation. Moreover, Tau mice, as a group, performed similar to Tg- controls on all behavioral measures analyzed during the test period. Tanemura et al., (2002) reported that V337M mice, which form hippocampal

NFT's as early as 11 months, show increased locomotion in the open field tasks and elevated plus maze and spent more time in the open arms of the elevated plus maze at 11 months of age. While these observations involve a different transgenic line and different age group from the animals used in my study, they still form a critical basis for comparison. As discussed earlier, the P301L mice evaluated in my study showed no differences in their level of activity or anxiety compared to Tg-. It must be noted, however, that the V337M mutation results in more extensive NFT formation and less profound cell death than the P301L mutation. Additionally, the V337M transgenic line produces NFT's almost exclusively in the hippocampal region, unlike the P301L line, which generates abnormal tau in the hindbrain and brain stem as well as the hippocampus, predisposing the animal to potential sensorimotor deficits. Tatebayashi et al., (2002) observed sensorimotor and cognitive function in 16-23 month-old R406W tau mutant mice, a transgenic line that produces forebrain NFT's beginning at approximately 18 months. In the study, Tatebayashi et al. observed that these mice display normal physical characteristics, sensorimotor reflexes, and motor coordination, compared to Tg-. These findings, albeit based on a different line of tau mutant at a different age, is in agreement with the results obtained in my study.

Effects of APP and Tau Transgenicity on Cognitive-based Tasks

To determine the cognitive deficits associated with the APPsw and P301L transgenic lines, transgenic and non-transgenic mice were evaluated in a variety of cognitive-based tasks. In Y-maze percent spontaneous alternation, APP mice were very nearly impaired ($p=0.07$) vs. Tg-, in their ability to spontaneously alternate between the

three arms of the maze at 8.5 months of age. Overall, the APP group was impaired, compared to Tg-, in the percent alternation task, while Tau mice show no significant deficits in this task. A study by Hsaio et al. (1996) indicate that APP mice are unimpaired at 3 months of age in Y-maze spontaneous alternation, but develop impairments at 10 months of age. These results agree with the findings from my study, indicating that APP mice are nearly impaired at 5 and 8.5 months of age and impaired overall for both time points. Also consistent with my findings, Holcomb et al., (1999) studied the performance of APP mice in the Y-maze spontaneous alternation task and found that they develop significant impairments at 6 months. A study performed by King and Arendash (2002a) revealed that APP mice develop deficits in Y-maze alternation as early as 3 months of age, but not at 9 months of age. King and Arendash do, however, report an overall Y-maze impairment from 3 to 19 months of age.

The Morris water maze acquisition test, which requires that mice learn the location of a static, submerged platform over a period of nine days, also revealed cognitive deficits in the APP group. In this task, the APP mice had a significantly longer latency, compared to Tg- and Tau, to locate the hidden platform on the final day of acquisition (day 9), as well as overall. Tau mice showed no impairments in locating the platform. The memory retention phase of this task (which occurred prior to acquisition testing on days 4 and 7, and following the last day of acquisition on day 10) evaluated mice on their ability to search for the escape platform after the platform had been removed from the pool. Animals that spend the most time in the goal quadrant, which formerly contained the escape platform, are said to demonstrate superior memory retention. While both transgenic groups performed similarly to Tg- on days 4 and 7, the

APP mice demonstrated a significant impairment in memory retention in the final retention trial (day 10) compared to Tg-. In addition, APP mice also exhibited an overall impairment in memory retention compared to Tau and Tg- groups across all three retention trials. These acquisition and retention impairments in APP mice cannot be explained by each mouse's swim agility, as all groups of mice were found to swim at approximately the same speed when placed in the pool. Hsaio et al., (1996) found that Tg2576 mice show no impairments in Morris water maze acquisition or retention at 2 or 6 months of age, but begin to exhibit such impairments at 9-10 months. The results of my study indicate that APP mice are, in fact, impaired in Morris water maze acquisition and retention as early as 5.5 months of age. It must be noted, however, that Hsaio et al. employed APPsw mice with a Tg2576 background, while my study involved APPsw mice with a more mixed strain background. Further, Westerman et al. (2002) conclude that Tg2576 mice develop impairments in Morris water maze acquisition and retention beginning at 6 to 11 months. In contrast, Koistinaho et al. (2001) report that APP mice are impaired in Morris water maze much earlier--as early as 3 months of age. In their comprehensive behavioral study of APP mice, King and Arendash (2002a) find that Tg+ mice possess no performance deficits in water maze acquisition and retention through 19 months of age, compared to Tg-.

APP mice were also found to be impaired in the platform recognition task, requiring animals to swim to a clearly visible, raised platform, whose location changes throughout the four trials of daily testing. Overall, APP mice required a significantly greater amount of time to locate and mount the visible platform than Tg-. Specifically, the APP mice performed worse in the platform recognition task than either Tau or Tg-

mice on the first day of testing, and demonstrated longer latencies on day 3, compared to Tg- only. Curiously, the APP group of mice did not demonstrate an impairment to locate the platform on the final day of testing, often described as the best indicator of task performance. This result may be an indication that APP mice are slow in their ability to change their search strategy. Unlike the Morris water maze, which requires that mice memorize the spatial location of a fixed hidden platform, the platform recognition task demands that mice be able to track the changing locations of a visible platform in the same pool. Tau mice were not significantly impaired compared to Tg- in this task. King and Arendash (2002a) found that Tg2576 mice are impaired in the platform recognition task at 9 months and beyond. My data indicates that impairment in platform recognition occurs much sooner in APP mice, as early as 6 months of age.

The radial-arm water maze (RAWM) was also used to assess cognitive function in this study. RAWM is a sensitive task that assesses working memory. In daily testing, working memory is most evident on the last of the four successive acquisition trials (T4) and the delayed retention trial (T5), which occurs 30 minutes after completion of trial 4. Unlike the Morris water maze, where the location of the platform remains constant throughout the nine days of testing, the RAWM task employs a semi-random sequence to rotate the platform between each of the six swim arms for each day of testing. This unique facet of the RAWM task is what requires the use of working memory. The RAWM task also requires spatial reference of external cues, as does the Morris water maze, to locate the hidden platform. When mice were evaluated across the 12 days of testing, APP mice were found to exhibit an "overall" impairment on T4 and T5 in locating the hidden platform, compared to Tg-, indicating working memory impairment.

When the 12 days of RAWM testing were analyzed separately in four 3-day blocks, APP mice were found to make more errors than Tg- and Tau in T5 for blocks 2, 3, and 4, as well as T4 in block 2. APP and Tau mice were also nearly impaired vs Tg- in T4 for block 4. Although not reported here, similar results were obtained for RAWM latency. Despite the wealth of information concerning the cognitive impairments of APP mice, there is no published data describing the performance of these mice in radial-arm water maze. A study by Arendash et al. (2001) did, however, describe the performance of APPsw+PS-1 mice in the radial-arm water maze task at both 5-7 and 15-17 months of age. The study found that these mice showed no impairments in T4 performance at either time point but did display impairments in T5 at 15-17 months.

Finally, circular platform, a cognitive task that incorporates stress and anxiety, revealed no significant impairments for either transgenic group compared to Tg-. King and Arendash (2002a) are in agreement with my results concerning the circular platform task, concluding that APP mice are unimpaired in this task through 19 months of age.

Currently, very little is known with regards to the cognitive impairments incurred by mutations to the tau protein. Tanemura et al. (2001) do report, however, that V337M tau mutants are unimpaired in the Morris water maze at 11 months of age. These results are in agreement with the findings described in my study, indicating that P301L tau mice, as a group, are unimpaired in all cognitive tasks. A study by Tatebayashi et al. (2002) found that R406W tau mutants are impaired in the contextual and cued fear condition tasks, measures of associative memory. Thus far, this is the only published evidence that any mutation to tau is capable of generating cognitive impairments in animal models of Alzheimer's disease.

Factor Analysis

Factor analysis is a very powerful tool used in statistics that has the ability to evaluate a large data set, determine which measures contribute the most variance to the data set, and what the underlying relationship is between these measures. In this study, factor analysis was used to group all 39 behavioral measures into common factors, each contributing to the variance of the behavioral data set. Each factor contains behavioral measures that are related to one another. Behavioral measures that are grouped into the same factor are therefore assessing a similar component of behavior (i.e.: sensorimotor function, working memory, etc.). Through the use of factor analysis, we can infer the relationship between multiple tasks, and predict how performance in one task might be predictive of performance in another task. In this study, two separate factor analyses were performed, one using the entire 39-measure data set, the second using a more selective 19-measure data set. The results of the factor analysis indicate that the RAWM working memory task, platform recognition task, and Morris water maze acquisition task share a lot in common, as they all load together under factor 1. These three tasks each serve as indicators of unique aspects of cognitive function, including working memory, recognition, and reference learning. Balance beam and string agility also loaded together, under factor 2 for the 39-measure analysis, illustrating how these two tasks both serve as measures of balance and agility. Y-maze spontaneous alternation and circular platform also loaded together (factor 3 of the 39-measure analysis), underscoring the cognitive-based properties of these two tasks. In each analysis, Morris maze retention loaded independently, indicating the unique nature of reference memory from all other cognitive measures.

While factor analysis is a fairly common statistical tool in various scientific and mathematical fields, it is still a novel concept in the realm of behavioral analysis. As such, there is little precedent for this type of analysis in Alzheimer's transgenics. While King et al. (1999) were the first to utilize factor analysis to determine which behavioral measures relate to one another, the factor analysis employed in their study was "rotated" and only task (not measure) loadings were reported. Based on my findings, therefore, I believe that factor analysis has the potential to further explain the complex relationships between the tasks currently used to evaluate sensorimotor and cognitive function in transgenic mice.

Discriminant Function Analysis

Like factor analysis, discriminant function analysis (DFA) is a powerful statistical tool that has recently been introduced into behavioral analysis. DFA works by evaluating multiple data sets to determine whether the data sets can be discriminated from one another and which measures are best able to distinguish them. In this study, DFA was used to evaluate the behavioral data from the three groups analyzed (Tg-, APP, and Tau) to determine whether they could be completely discriminated and, if so, which behavioral measures can best discriminate between the three groups. Two DFA methods were used in this study, the direct-entry method and the stepwise-forward method. In addition, for each method, two separate analyses were run, one with the entire dataset, and the other with only 15 behavioral measures. The direct entry method utilizes all behavioral measures in the DFA model, while the stepwise-forward method selects measures one at a time, keeping only those variables that are best able to discriminate between the three

groups in the final model. The results of the DFA analysis indicate that the direct entry method was unable to discriminate between the three transgenic groups. The stepwise forward method, however, was able to discriminate between the three groups using only eight behavioral measures. The 15- and 32-measure stepwise DFA models each arrived at a set of eight measures, with both sets having most of these behavioral measures in common. In both analyses, cognitive measures, including RAWM working memory, Morris water maze, and platform recognition, were found to be critical in discriminating between the three groups. Certain sensorimotor tasks, such as open field activity, balance beam, and string agility, were also found to be important in discriminating between Tg-, Tau and APP groups.

DFA, like factor analysis, is still a very new concept in behavioral analysis and, as a result, has no precedent in Alzheimer's transgenics. Like factor analysis, however, DFA appears to hold great promise for inclusion in future behavioral studies. DFA has the potential to eliminate the need for large behavioral test batteries, instead selecting a small subset of behavioral measures that are equally capable of discriminating between various transgenic groups. In this way, less time and effort would be needed to accurately evaluate the sensorimotor and cognitive function of transgenic mice and the effects of therapeutics therein. Before DFA can be used with great accuracy in a wide variety of behavioral studies, however, it must be studied further to determine how its precision holds up with different transgenic lines and various age groups. In the future, DFA may be instrumental in the development of better behavioral test batteries.

Correlations Between Behavioral Performance and Tau Pathology

In order to create a link between the biochemical and pathological changes that take place in the brains of tau mutant mice with their associated sensorimotor and cognitive deficits, a correlational analysis was performed. In the analysis, five measures of tau pathology were used as biochemical markers: number of tau+ neurons in the brainstem, cortex, hippocampus, amygdala, and whole brain. These five pathological measures were entered into a correlational matrix with the 39 behavioral measures discussed previously. The results of the analysis indicate that tau pathology is exclusively correlated with deficits in cognitive function, particularly in the swimming tasks. An increased number of tau+ neurons in the whole brains of P301L mice was found to be correlated with impaired performance on the final day of Morris maze acquisition and platform recognition, as well as overall performance in platform recognition. When the number of cortical tau+ neurons was correlated with behavioral impairments, these same relationships were seen, indicating the critical nature of the cortex in cognitive function. The amount of hippocampal tau was found to be correlated with performance in the platform recognition and radial-arm water maze tasks, specifically T5 errors in the final block of testing and overall T5 latency, two measures of working memory. This finding seems to illustrate the function of the hippocampus in working memory and spatial acquisition. There were no significant correlations between behavioral performance and tau pathology in the brainstem and amygdala, indicating the relative unimportance of these two brain regions in cognitive function. While brainstem tauopathy has been linked to sensorimotor deficits in other Tau studies, the relative lack of any such correlation in this study, coupled with the absence of any significant sensorimotor deficits, indicates

that tauopathy present in the brainstem during the behavioral testing period was not detrimental to cognitive or sensorimotor performance.

As discussed previously, there are only a small number of studies that have attempted to characterize the behavioral impairments associated with tau mutations. Of the few studies that have been carried out, none of these has utilized a comprehensive test battery to assess sensorimotor and cognitive deficits. As a result, there is no precedent for the type of correlational analysis that is described in this study. Based on these findings, however, we can conclude that a strong correlation exists between the formation of NFT's in the hippocampus/cortex and behavioral impairment. The fact that the P301L mice observed in this study failed to show any significant cognitive or sensorimotor impairments between 5 and 8.5 months of age illustrates that these mice, as a group, did not develop extensive enough NFT formations during the age range evaluated to generate such impairments in all mice. However, NFT formation in individual animals (those further along in forebrain tauopathy), was associated with, and likely causative to, cognitive impairment in three tasks. Unfortunately, the presence of extensive NFT's in the hindbrain and brainstem of these P301L mice results in premature hind limb paralysis and death, precluding the assessment of behavioral impairment in these mice beyond 9 months. If such an evaluation were possible, however, it would be expected that P301L mice, as a group, would become significantly impaired in cognitive tasks beyond 9 months of age, corresponding with an increase in the number of cortical and hippocampal NFT's.

In summary, the results of this study indicate that APP mice are cognitively-impaired at a young age, as early as 5.5 months old, while appearing to be largely devoid of any debilitating sensorimotor deficits through the same time frame. Conversely, tau (P301L) mutants are found to have no significant sensorimotor or cognitive impairments through 8.5 months of age.

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